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OPENING SPEECH

By

K. H. BAAGØE,

President.

Ladies and gentlemen,

As President of the First Northern Congress of Allergy I extend a hearty welcome to all of you. We have been especially pleased that so many colleagues from our brother countries have accepted the invitation. In fact, the large attendance bears witness of the interest taken in the problems of allergic research.

As an introduction I shall attempt to give a brief survey of the beginning of the study of allergic diseases in Scandinavia.

The first to raise the question of allergy in connection with hayfever-asthma diseases was the Norwegian *Arent de Besche*. Arent de Besche is no longer alive, he died in 1945, 67 years old. So I may be allowed to say a few words about his share in allergic research.

As early as 1909 there issued from his hand an account of a case of horse-asthma in which an injection of diphtheria-serum caused a violent attack of asthma. Arent de Besche interpreted this as an anaphylactic shock and stated that the shock was followed by an antianaphylaxis lasting for three months.

The next work appeared in 1918 and was followed by a number of treatises on allergic subjects, such as asthma, hayfever, passive transmission of allergy to animals and to normal human skin; he also published some studies of genealogical tables of allergic families.

De Besche discovered the ophtalmo-reaction in asthmatics

with allergy to animals, as Blackley had demonstrated the allergy to pollen in hayfever patients. The reaction was brought about by stroking the fur of the animal with a finger and then touching the patient's conjunctiva. He himself called it "de Besche's ophtalmo-reaction."

De Besche was also the first to succeed in transferring the allergy of an asthma-patient to normal human skin. At the time when he made this experiment he was not acquainted with Prausnitz and Küstner's account of the transmission of allergy from a patient with alimentary idiosyncrasy to fish and eggs in a similar way.

De Besche did an important piece of work in allergic research. I would like you to stand up together with me in memory of Arent de Besche. We shall remember him as the pioneer in the Scandinavian countries within the province of allergy research work.

In Denmark the problem of allergy was taken up in the beginning of the twenties at the Children's Department of "Rigshospitalet," whose head was then Professor C. E. Bloch, from whose department my treatise on asthma appeared in 1926.

In Sweden there appeared in the twenties some minor papers on allergic subjects by *Tillgren* and *Sture Berggreen*. But soon *Ernst B. Salén* took the lead. Even in his first work (1932) he established that for the treatment it was absolutely necessary to make an exact etiological diagnosis, to which cutaneous reactions were an important aid. In the same paper he published a series of experiments made a. m. Prausnitz-Küstner-de Besche, by which he proved that a local reaction could be brought about not only by injection of the allergen into the skin, but also by administering the allergen endonasally, perorally or per rectum. Salén concluded from this that the allergen can pass through the normal intestinal membrane, and he was thus in sharp opposition to Hanhart and Karl Hansen, who maintained that a pathologically increased permeability of the cells in the allergic patients was an essential condition for the absorption of the allergens.

In collaboration with Juhlin-Dannfelt, Salén has found further support for his view by their investigations into "latent allergy."

In Finland allergic research work was taken up with great energy by Mrs. *Eriksson-Lihr* and others in the early thirties.

In Iceland no work has yet been done in this sphere.

If we look back to the first days of allergology to see what the introduction of the concept of allergy into asthmatology meant in our country—and I suppose it was the same in the other Scandinavian countries—the first impression may be that it was a disappointment.

When it had been proved that a specific hypersensitiveness could be discovered by cutaneous reactions, many physicians thought that now the problem of asthma was solved: It would only be necessary to scratch the patient's skin with some substances and eliminate these substances from the environment or food of the patient—and he would be cured. This did not prove true, hence the disappointment.

But even if relatively few patients could be helped by pure elimination, I would like to call attention to the fact that the introduction of the concept of allergy proved important in another way.

At that time—about twenty to twenty five years ago—it was the general opinion that asthma in children and young people was a neurosis, and when a mother came to the doctor with her child, she was often told that asthma was due to "nerves," and that the treatment was mainly a matter of the child's up-bringing. This again meant that the parents were not to take the child's fits too seriously, they were to take as little notice of the child as possible, and the father and mother who took their task seriously treated the child's attacks of asthma with scoldings or perhaps beatings, and if the child happened to have an attack in school, the conscientious teacher did the same, the child was reproved and put in the corner.

In short, in addition to suffering from what Willis calls "morbus maxime terribilis" the child had to suffer the injustice that the disease was regarded as hysteria, which to the layman is equivalent to affectation.

In this state of affairs the establishment of asthma as an

allergic complaint caused a great change. It certainly took some time, but by degrees the view prevailed that asthma is not a hysteric disease. And to us who have known the old times, it is a satisfaction to realize that to-day there is hardly a doctor in the country who will send away a child with the information that it is only "nervousness."

In the past years great work has been done in Scandinavia to improve the conditions of asthmatics and to find the best treatment of asthma and other allergic diseases.

I hope that this congress will contribute to deepening our knowledge of the nature of allergic diseases, and that it may give all of us inspiration to further research, the ultimate aim of which is to help our patients.

With this wish I declare the First Northern Congress of Allergy opened.

Acta allergologica, 1948, I, 127—166.

THE DIAGNOSTIC AND CLINICAL IMPORTANCE OF THE SKIN TEST[1]

(from the viewpoint of internal medicine)

By

ERNST B. SALÉN

A short historical account and general orientation.

The honour of having first ascertained the possibility of determining through a skin test whether a person is specifically *allergically* hypersensitive to a certain substance should perhaps be divided between the Englishman Charles Blackley and the American Norill Wyman, whose works on the subject were published at the beginning of the 1870's. Both authors treated of the inoculation of pollen into the skin, the former pollen of the common grasses, the latter pollen of Ambrosia. By means of comprehensive researches extending over almost twenty years, Blackley had succeeded in establishing the method's specificity to common kinds of European grass pollens. He was also the first to make the ophtalmoreaction, whereby he established parallelism between the specific state of hypersensitiveness in skin and certain mucosae. Blackley also made a series of exposure and provocation-tests on healthy people and persons with hay fever, as well as elimination tests of the latter group. The results were such that they must be taken as *proof* of the clinical significance of pollen. These so important investigations of Blackley's must without doubt be characterized as *fundamental to the whole of modern allergy diagnostics.* Against this background it becomes somewhat hard to understand that early in the 20th century there were scientists, who were still making efforts to trace and even believed they had found the pathogenic microbe of hay fever, while others stubbornly stuck

[1] Opening address to the Congress.

to the old theory of neurosis. The reason was, of course, that the time had not yet come for accepting and, more widely speaking, practising these interesting results of Blackley's pioneer research work on a broader front. It was not until Richet's discovery of anaphylaxy and Pirquet's ingenious conception, which was partly founded on that discovery and through which the clinical notion of allergy was created, that the road to its further development was opened. It was of fundamental importance in this connection that American authors (Chandler Walker, Coca, Cooke and others) were able to demonstrate how the same test methods which Blackley had adapted to pollinosis, could be used for a number of other forms of allergy, with the result that Robert Cooke introduced the allergy test as the routine method about 1911. Almost at the same time the Englishmen Noon and Freeman (1910-11) were able to demonstrate the possibility and value of *specific* desensitization in cases of pollen allergy, a mode of treatment which it was later on found possible to utilize with the same success in connection with other forms of hypersensitiveness. Finally in 1909 Bruck successfully completed his experiments with *heteroloous* passive transfer, and in 1921 and 1922 Prausnitz & Küstner and de Besche their positive experiments with passive *homologous* transfer, whereby the presence of *specific* antibodies or reagins in the blood of allergics was established, and through which was likewise created a clinically applicable method of establishing the *serological* specificity of the direct skin reaction. As the last important links in the chain of development came Landsteiner and his co-workers' creation of the hapten doctrine, which embodied an explanation of the formerly obscure fact that simple chemical substances of a non-proteinous nature might, under certain conditions, cause *sensitization* and *excite* symptoms of allergy; and, finally, Dale's doctrine of a histamine-like substance as the active principle in the occurrence of the allergic shock symptom, a doctrine which has made an explanation of *physical* allergy possible and allowed us to co-ordinate in principle this mechanism with the mechanism of protein and hapten allergy.

I have taken the liberty to give this short historical account to show how the conditions for the clinical application of modern

allergy diagnostics seem to have been at hand long ago. An attempt to introduce antigen analysis as a routine method was even made here in Sweden in the beginning of the 1920's. The results, however, were little encouraging and the experiments were discontinued early. We can now state the reasons with a fair amount of certainty: the extracts available at the time were not always reliable, and in addition the series of extracts was very defective, i. a. we lacked the dust extracts that are of such practical importance in Sweden, and also bacterial extracts. On the whole, in ointment form and with the tests made by the scarification method these extracts were not very suitable. These unsuccessful experiments of the 1920's must have left deep marks behind them, and there is no doubt that many of the hospitals in which they took place never got over the scepticism aroused by their results. However, it is probable that several other circumstances added to this scepticism. As so often happens, the new diagnostic methods tended to be overrated, and it is not until later on that the limitations of their clinical value, which necessarily must be taken into consideration, were better defined. In the following I shall revert to this question. Another important circumstance, which was probably also prejudicial to the development, is referable to the allergen extract itself. For the allergen world was rather sparsely populated, and it could only be enriched by stages. Not infrequently an approximately complete skin test now requires the use of a vast number of different extracts. If in such test some causal allergen or other is missing, the result may easily be taken as an argument for the clinical worthlessness of the method. No doubt this must have been the case many a time. The same holds good of the preparation, testing and standardizing of the extracts. That imperfections have often occurred and are still not infrequent, must be taken as a matter of course. Of this fact I shall also later on give some instances.

A final circumstance, which has probably also contributed to a sceptical attitude towards modern allergy diagnostics, is that when used as a basis for clinical procedure it often failed to yield the expected result. The causes have undoubtedly been numerous and varied. Clinical allergy grew forth from the beginning as an

almost entirely isolated speciality, which by no means always retained *that intimate connection with internal medicine altogether which must be regarded as a necessary condition for successful progress.*

A circumstance which was not always taken into account was that in the majority of allergic cases a number of *different* causal factors might act together, some of a purely allergic nature and some of a different kind, the diagnosis and treatment of the latter requiring the application of the principles of internal medicine as a whole. The usual and important influence of infection complications on the allergic action was for a long time not taken into consideration, or at least only partly so. The existence of bacterial or toxic allergy has only recently been recognized in some measure. The same holds good of a number of other factors. It is therefore of importance to emphasize how the functional antigen analysis should only be interpreted and classified as a diagnostic *adjuvant* in the allergic diseases, and that all cases of such disease should moreover be submitted to *a careful internal-medical examination and judgment.* These points of view I have already voiced at the Scandinavian Congress of Internal Medicine at Upsala in 1933, when I also quite distinctly underlined the great importance, direct or indirect, of existing infection complications to the allergic action and the clinical procedure.[1]

In conclusion, an indication of very great importance to the question under discussion to-day. Earlier the science of allergy concentrated on the approachableness of the allergic to sensitization, and presupposed that, if such sensitization were established and followed by the necessary exposure, it *must necessarily* lead to clinical manifestations. When in a skin test now and again strong reactions with certain allergens were obtained and no evidence of the causal importance of the same allergens could be traced through exposure or elimination experiments, the natural result was that this was taken as a proof of the unreliability and worthlessness of the skin test. It was only with the demonstration of the not unusual occurrence of a *latent* allergy and the

[1] See Salén 2, and in Nordisk Medic. Tidsskrift, 1932.

simultaneous statement of the serological specificity of these "latent" skin reactions by positive transfer experiments (Salén and Juhlin-Dannfelt), that the apparent disavowal of the reliability of the skin test by these positive skin reactions was abolished, and at the same time these experiments forced an important revision of one or two of the fundamental principles of the science of allergy, i. a. the thesis of the abnormal permeability of the mucosae of allergics.

In summing up this short historical review, it might be said that from both a technical and a clinical point of view modern allergy diagnostics from the beginning have been burdened with a number of defects and weaknesses, which make it quite understandable that the reliability and value of these diagnostics were questioned or met with doubt by many people. In the following I shall try to give examples in support of what I have said and through such examples try to prove whether it has been possible to eliminate such defects and weaknesses through the progress in later years, and if so, to what extent.

From the above now emerges a fact which deserves to be pointed out particularly in this connection. Henceforth, no one who is familiar with the problems of allergy will surely dispute the justification of placing the allergic diseases in a well-defined and separate group. But it must then be remembered *that it was the experience reaped from the allergic skin test which, from the beginning, formed an important part of the basis of this clinical conception* and made it possible to merge into a homogeneous clinical group of diseases such diverse phenomena as asthma, rhinitis "sympathetica," most forms of urticaria and Quincke's oedema, some cases of migraine, certain cases of gastric and abdominal symptoms and certain cases of eczema and dermatosis etc. Already this fact must in my opinion be entered on the credit side of antigen analysis.

The Concept of "Allergic Diseases."

A critical study of the concept of "allergic diseases" is called for here. It stands to reason that the fundamental condition to

be complied with if the allergic skin test is to be applicable and useful, must be that the morbid condition does actually express an interplay between an allergen (antigen) and corresponding antibodies, in other words: that the clinical symptoms are governed by an antigen-antibody reaction in the organism. It is now obvious that this fundamental condition is non-existent in a number of cases which are clinically groupable within the concept of allergic diseases. As far as the first group is concerned i. e. that of exogenous protein allergy, we have sufficient evidence that the fundamental process really is of an antigen-antibody nature. Here a positive result of the skin test may thus be expected, as well as of the experiment with a passive homologous transfer. Concerning group No. 2, "endogenous" allergy, it is probable that the same holds good of certain of the forms included therein. In the case of menstrual allergy, for example, both a positive direct reaction and positive transfer are obtained with suitable extracts. (Salén 3). In thyroid-adenoma allergy both have been positive (own observations). In cases of bacterio-toxic allergy it is probable that a hapten mechanism is usually active. With van Leeuwen's bacterial extract a positive, usually direct ("immediate") reaction may sometimes be obtained, but frequently only a delayed reaction of the positive Mantoux type. Transfer tests are not successful as a rule.[1] In keeping with the familiar pattern, group 3 has been placed under the heading of drug allergy. However, I wish to emphasize that this heading is not adequate. American handbooks usually speak of medicinal or drug allergy as a separate group, the purpose being to express the circumstance that the allergizing and exciting substance, which thereby becomes causal, is of a hapten nature, from which follows that both skin test and transfer test will be negative. As I have previously had occasion to show, this grouping, however, does not agree with the actual circumstances. For a number of drugs, such as digitalis, glycyrrhizae, senna, senega, lycopodium a. o.[2] it is a fact that skin tests as well as transfer tests give a clearly positive result. If we take more

[1] Only in two cases of Prûrigo Besnier we have seen a positive reaction.

[2] See Salén 4, 9 and 5.

simple chemical substances, we will find that we shall obtain a positive result of the direct skin test as well as the transfer test with for example chloramine, persulphate of ammonia etc.[1] The whole of this group should therefore rightly be categorized in a different way. It should be possible to define it as follows: it includes cases of allergy where the causal substances are typically hapten in nature and action, whereby the direct skin test as well as the transfer test are negative. The criterion of nevertheless categorizing the process as *allergic* and of interpreting it according to Landsteiner's hapten doctrine is that the clinical symptoms, which with the particular substance in these cases may be provoked, do *not* appear as the expression of an habitual intolerance of the substance, but take the form of an allergic symptom-complex, Quincke's oedema, urticaria, asthma, itching, scarlatiniform eruptions, eczema, conjunctivitis with ocular oedema etc. Only a definition such as this will make the separation of such a group sound.

With physical allergy, a very small group, we leave real allergy, we do not have to consider an antigen-antibody reaction as fundamental, or consequently, the occurrence of a positive skin reaction or transfer test. In fact, with the investigations of Dale and others as a basis, we might ascribe the clinical manifestations also here to the action of a histamine-like substance.

The psychogenically conditioned allergy is doubtful and unclarified in both existence and mechanism. As far as asthma is concerned, we may say that it is the shrunken remnant of the once so paramount conception of Asthma "nervosum." Vaughan, who took a special interest in this question and, besides, had behind him an unusually wide experience in the field of allergy, is doubtful about the existence of a purely psychogenically conditioned allergy, but has no doubt that a psychogenic excitant may occur in persons suffering at the same time from a genuine allergy.

Concerning this division into groups, however, I would point out one thing which is not without importance to the question under discussion. The division of the true cases of allergy into

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[1] See Salén 4, 9 and 5.

the above three main groups (i.e. the three first mentioned) is artificial to a certain extent and does not wholly correspond to the actual conditions. A case belonging to the first group, i.e. with clear, direct reactions and transfer tests, may likewise be hypersensitive to a certain hapten, and may consequently also be placed to group 2 or group 3. A case belonging to group 3 may also have a bacterial or toxic allergy and thus belong partly to group 2. As we shall show in another connection at this congress,[1] this is true of almost all cases of aspirin hypersensitiveness.

From what I have said it will thus appear that functional antigen analysis has a limited range of application within the clinical group of allergic diseases. I shall later endeavour to give a few statistical facts which throw light upon this and also give an opportunity to show *how the percentage of the occurrence of positive skin spectra is much displaced according to the nature of the material on which the calculation is based.*

The nature and testing of the allergen extract.

It is of course of fundamental importance to the value of the skin test that the reagents employed—allergen extracts—should be absolutely reliable and of full value both qualitatively and quantitatively. As the question of the precautions which should be observed in the assembling of the raw material and its handling, as well as the procedure in preparing the extract, will be described in every detail at the proceedings to-morrow (E. Bruun), I shall restrict my comments to-day to emphasizing the eminent importance of these questions to the whole subject of diagnostics. As far as we ourselves are concerned, we have as a rule followed the directions of Storm van Leeuwen, but with certain modifications of the principles laid down in the statement made by Wittich.

However, I feel obliged to dwell somewhat longer upon the question of standardization. The procedure mainly employed in the U.S.A. is, as we know, to standardize the extract by determin-

[1] Salén and Arner, Acta Allergologica, Vol. I, 47, 1948.

ing either the total nitrogen content (Cooke and Coca), the Protein Nitrogen Unit (Cooke and Stull), the molar unit, or of the Delapan unit. The standardization of pollen extracts is, as we know, usually based on the Noon Unit or Dilution by Weight. During my first years of occupation with these matters I had the opportunity to try a number of the various methods. As my experience has been that even if the most accurate chemical methods of determination are followed, there is no certainty of determining even the presence of nitrogen (Jorpes) in a highly biological allergy extract as for instance of lycopodium, it seems to me that the sceptical attitude we have long assumed towards the value and reliability of these methods of standardizing must be regarded as highly justifiable. Instead we have therefore for the last fifteen years made use of the biological method of standardizing according to the directions of van Leeuwen, that is to say, we have tested the extract on a number of non-allergics (10-20) with—usually after a necessary dilution—a negative result, and for practical reasons used the obtained extract solution diluted 1 : 10, and also tested and usually also titrated one or more allergics whose hypersensitiveness to the allergen in question had been demonstrated and titrated earlier. This seemed to us to be the surest way to obtain reliable extracts. If careful titrations are made it becomes possible, by comparing the old extract to be replaced and the new, to establish approximate agreement in the question of their biological activity, a very important desideratum in connection with specific desensitization. That defectively standardized extracts are not infrequently used, and that the consequent conclusions and the therapeutic procedure must necessarily be radically faulty, are facts which it should be possible to illustrate by well nigh drastic examples. It is clear that such results must rightly arouse distrust as to the reliability of the antigen analysis. In this connection it must likewise be pointed out that a number of extracts will remain stable only for a limited time and therefore a *continued* check on their strength is necessary. It seems to me *that all these facts necessitate that preparation, standardizing and continuous control should take place in a clinic for allergic cases.*

Technique and Evaluation.

Concerning the technique of the skin test on the whole, I shall emphasize the desirability, indeed the necessity, of constantly using the same hypodermic syringe for every extract.

In the question of the method of the skin test we have long used the intracutaneous, whereby about 0.05 ml. of each extract is injected as well as of the positive (histamine 1 : 10000) and negative control (carbol-common salt). The test is made in one seance, in the back as a rule. The intracutaneous test is about 100 times more sensitive than the scratch test and, in our opinion, much easier to make and judge. The risk connected with the intracutaneous test is relatively small, as far as our experience goes. In about 10,000 cases of allergy tested in this way we have seen more perceptible shock symptoms in some fifty instances, and of those only some ten were of a graver kind. In no case was there a mortal termination. Some reserve with regard to the above is, however, necessary. If the patient during the recording of his history or filling up of the questionnaire has given such information that a rather high grade of hypersensitiveness to a certain allergen is probable, that allergen is excluded in the back test and a test of it is made separately, either an intracutaneous or a scratch test, but in a part of the body that can be tied off with a piece of tubing or a tourniquet, as for instance the forearm. If an obviously strong reaction occurs—the sign is that it develops particularly speedily, gives pseudopodium-like blisters round the periphery—or a shock symptom, we immediately apply a tourniquet and inject adrenaline, *partly into the floor of the plaque, partly into other parts not stased,* and if required an intravenous calcium injection as well. The assisting nurse should have standing orders to observe the reaction field at short intervals and to give warning immediately if anything unusual should happen. Adrenalin and calcium should always be at hand ready for injection.

In the question of judging the degree of hypersensitiveness, it is very tempting to assume and believe *that on the basis of the strength of the primary reaction it should be possible to arrive at*

an almost reliable conclusion as to the degree of sensitization. Such an assumption is of course justifiable within certain limits, *but by no means altogether.* It is not infrequently found that primary skin reactions of almost the same strength correspond when titrated in the one case to a titre of 1 : 1000 or 1 : 10000, in the other case to 1 : 10 millions. Recognition of this is of course of enormous practical clinical importance, the consequence being that the dosage schema for the specific desensitization can never be drawn up on the basis of an approximate estimation of the degree of sensitivity and guided by the strength of the primary skin reaction, *but must always be based upon a careful titrating of the degree of hypersensitiveness.*

If the precautions mentioned are observed, it is our experience that the risk of the occurrence of more serious shock symptoms is no greater than that connected with a specific desensitization. If such symptoms should arise it is, however, of importance that the patient should remain for some hours in the hospital or in the reception ward after the adrenalin or calcium has been administered. After the therapeutic effects of these remedies have passed, it has happened in isolated cases *that the shock symptoms reappeared and thus required repeated treatment.*

In passing, I would emphasize *that adrenalin or any related preparation should not be given to the patient 6 to 7 hours before the skin test is made*—a precaution which seems to have received little attention in the literature—as, if this precaution is not observed, it seems that a positive reaction might be inhibited, as a result of which important signs may be overlooked in practice. I shall not yet venture to express any opinion as to whether the more recent anti-histamine preparations have a similar effect, but our experience hitherto argues rather against such a possibility.

In reading the skin test it is of course a primary requirement that the negative control (carbol-common salt) is clearly negative, the positive (histamine 1 : 10000) just as clearly positive. As far as our experience goes, exceptions are very rare. In the case of an unclear negative control, however, it is generally still possible to use the skin test, i.e. when the difference of degree between the negative control and a definite allergen reaction is fairly great.

If there is still a doubt in the evaluation, as might happen in exceptional cases, *this doubt is simply and surely eliminated by making an indirect re-test in the form of an experiment with passive homologous transfer*, an expedient which seems to have received too little attention. A negative histamine control as already mentioned is only obtained by way of exception, and mainly in elderly and cachectic persons. As it has been maintained in various quarters that allergics relatively often have an abnormally intensified *non-specific* skin-sensitivity—which is said to occur predilectively in children—I must make the objection that to the best of my knowledge no objective and acceptable investigation into this matter has been published. By a mathematical treatment of the sizes of the histamine reactions, in a group of non-allergic and also in a group of allergic children, we have found (Salén, Hulting and Nordenfors) that in the case of both groups, almost corresponding values resulted for the size of the erythema as well as the plaque, and that consequently no statistically demonstrable difference in the question of non-specific skin sensitivity was found in the two groups. (Table 1.)

TABLE 1

Histamine reaction.

	Average diameter of Erythema in mm	Average diameter of plaque in mm
Asthma group	40.7 ± 2.11	14.2 ± 0.47
Healthy	37.9 ± 1.33	13.1 ± 0.31
	2.8 ± 2.49	1.1 ± 0.56

When reading the skin test earlier we used to record the exact measurement of erythema and plaque; but guided by experience we have since followed the practice of marking the degree of reaction with one, two, three or more plus signs, where a plus signifies a plaque of a greater diameter than 10 mm., two, one of more than 15 mm., three, one of more than 20 mm. For the material we shall describe in the following the strength has usually been *determined by titration*.

Choice and Amplification of the Extract Series.

It is of course of the greatest importance that the antigen analysis should be carried out with all such allergens which, having regard to the history and the answers to the questionnaire, and with due regard to the occupation, habits and environment, might be supposed to be of causal significance to the particular patient. A satisfaction of these conditions naturally requires a penetrating examination of all aspects of the case and not infrequently proves to be rather a time-wasting procedure. Especially in Prurigo Besnier the number of the tests may easily amount to a hundred or more.

I shall illustrate this by means of a few examples. On a number of asthmatic children (at the Arstahemmet) we have made tests with extracts of dust from the homes of each of the several children. Table 2 shows how a number of the children show a strongly positive reaction to *one* dust extract which for a different number of the children gives only relatively weak reactions, while later on strong reactions are obtained with one or more of the other dust extracts. *From this emerges the necessity that the routine test should include not only one but several different dust extracts, and in such a selection that they so to speak cover the whole field of dust allergy.*[1] It is not infrequently an advantage to use what we call *auto-dust*, i.e. a dust extract from the patient's home. In case this procedure is not followed, we shall easily lose a number of cases of dust allergy, incomparably the most frequent form of allergy in Sweden.

In this Table 2 we have included a case not belonging to the childrens' group, a foreman working in very "dusty" surroundings. In the routine test of this patient all dust tests were negative and, as will be seen from the table, the same was the case with all dust extracts used. In the meantime I had made further inquiries about the occupation of the patient and found out that his work consisted in making buttons of the so-called "stone nuts".[2] The polishing of these nuts causes a cloud of fine dust to spread in the room. With an extract of the nuts the patient gives an extremely strong local reaction, which even produced a severe asthma paroxysm. The Prausnitz-Küstner test was just as strongly positive. On testing the group of children with the same extract, negative reactions were apparently obtained on the whole (Table 2).

[1] Storm van Leeuwen's important contributions to the elucidation of the causal signification of dust allergy to asthma and rhinitis cases in Europe must be specially pointed out in this connection.

[2] A kind of palm fruit.

TABLE 2

Skin tests at the School Home, 23rd October 1940.

Tests with auto-dust extract from:

	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	Foreman*
1. L.	+	+(+)	+	+	++	+	++	—
2. W.	++	++	+(+)	++(+)	++(+)	+	+(+)	—
3. E.	++	++	+(+)	+++	++(+)	+	++	—
4. D.	++	+(+)	+(+)	+++	+(+)	+(+)	+(+)	—
5. E.	++	++	+(+)	+++	+(+)	+	++	—
6. S.	++(+)	++	+(+)	++(+)	++	++(+)	+(+)	—
7. S.	++	+(+)	+(+)	++(+)	++	—	+(+)	—
Foreman D.	—	—	—	—	—	—	—	+++

* Extract of dust from face-guard.

A second case: A printer was being treated for allergic rhinitis and asthma which, judged by the result of the routine test, seemed to have been caused by a fairly severe dust allergy. The patient improved under the treatment; a partial dust clearance was later made and the nocturnal manifestations disappeared completely. However, time after time the patient reported that he was continually having considerable trouble during his work, and that it came about when a certain fluid was used in the workshop (for fixing). An analysis of this fluid showed that it consisted of gum arabic dissolved in two kinds of alcohol. With an extract of gum arabic a maximal reaction was obtained and a severe Status asthmaticus set in. An experiment with passive homologous transfer was positive. Accordingly the case was that of a combined dust and "printer's" allergy. In passing we shall point out how easy it is to overlook things in such cases and to arrive at faulty conclusions both in connection with the value of the antigen analysis and that of the anti-allergic treatment measures.

A third case: This was the proprietor of a small mechanical workshop, where metal was cast in wooden moulds. He suffered from moderate asthma and said that his occupational environment exacerbated it. At the routine examination we were surprised by a strongly positive lycopodium reaction such as we rarely find outside the hairdressing, drug or theatrical occupations. When asked whether he had been employed in any of these occupations his answer was negative, but shortly after, when we were discussing the discovery with a colleague and mentioned the word "nikt" (lycopodium), the patient broke into the discussion and explained that almost every day he was highly exposed to the inhalation of lycopodium in his occupation. For lycopodium was used to prevent the metals from sticking to the wooden moulds.

These are only a few examples[1] of how important and difficult it is to include every imaginable causal allergen in the antigen analysis in every case. It is almost certain that this condition cannot always be satisfied. Most inaccessible for a complete analysis are of course the combined cases, where one might find for instance a strong dust allergy at the routine test, and let it rest at that. Such an oversight may easily lead to situations that might be deemed to discredit allergy diagnostics altogether.

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[1] A greater number of similar special cases have earlier (1946) been accounted for by the Swedish Society for Allergy Research (i. a. cases of linseed-, chloramine-, lycopodium-, H_1N-persulphate-allergy etc.). See also Salén 2-5.

TABLE

Intracutaneous skin-test of 36 asthmatic

Name	Age, years	Heredity	Eosinophil %	Control	House dust	Mites	Bed-feathers	Mould	Human Dander	Bacteria	Histamine 1/10000
M. L.	14	+	10.5	—	+(+)	++	+(+)	—	—	—	++
K. K.	7	0	10.5	—	++	+(+)	+(+)	—	+	—	++
H. J.	12	0	5.5	—	—	—	—	—	—	—	+
M. F.	12	+?	9.5	—	++	+(+)	+(+)	—	+(+)	—	+++
K. E. K.	8	+	13.5	—	—	—	—	—	—	—	+(+)
T. K.	9	0	8	—	++	++	++	+(+)	—	—	++(+)
H. J.	14	+?	8.5	—	+(+)	+	+(+)	+	—	—	++(+)
K. O. E.	9	0	9.5	—	++	+(+)	+(+)	—	—	—	++(+)
B. A.	9	+	12	—	—	—	—	—	—	—	++
S. B.	12	+?	8.5	—	++(+)	+(+)	++	+	—	—	++(+)
O. D.	12	0	10	—	++	+	++	+(+)	+(+)	—	++(+)
R. E.	7	+	6.5	—	+++	—	+(+)	—	—	—	++(+)
V. W.	11	+	3	—	—	Schw +	—	+	—	+(+)	++
B. W.	9	+	11.5	—	+	+	+	—	—	—	++
S. W.	12	+?	16	—	++(+)	++	+	+	+	—	++(+)
E. N.	8	++	4	—	+(+)	+	+	—	—	—	++
T. S.	8	+	6.5	—	++	++	++	+(+)	—	—	++(+)
B. N.	8	+(+)	7	—	+(+)	+	+	—	—	—	++
B. M.	10	+?	10	—	++	+(+)	+(+)	—	—	—	++(+)
B. O.	13	+?	3.5	—	++	—	—	—	—	—	++
R. L.	13	+?	5	—	—	+	—	—	—	—	++(+)
L. H.	13	0	4	—	++	+	+	—	—	—	++(+)
U. B.	11	+	5	—	++	+	—	+	—	—	++
G. E.	10	0	1	—	++	—	—	++	—	—	++
I. S.	12	+?	1.5	—	+(+)	+(+)	—	+(+)	—	—	++
I. R.	8	0	5	—	—	—	—	—	—	—	+++
Sv. B.	11	0?	23	—	+(+)	—	—	+	+(+)	+(+)	++
V. K.	12	+	12	—	++	—	—	—	++(+)	—	++
N. J.	10	0	8	—	—	—	—	—	+	+(+)	+(+)
B. A.	8	0	8	—	++	—	—	+	—	—	++
E. P.	12	+	5	—	++(+)	—	—	—	—	—	++(+)
K. A.	10	0	8	—	+++	—	—	—	—	++	+++
M. B. E.	9	0	8	—	+++	+	—	+(+)	—	+	++(+)
M. J.	8	+?	10	—	+(+)	—	+	+	—	+(+)	++(+)
S. E. J.	11	+	11	—	++(+)	—	—	+(+)	—	—	++
K. P.	13	+	5	—	++	—	—	—	—	—	+(+)

3 /

school children (16th November 1935).

Fish	Meat	Vegetables	Dog	Cat	Horse Dander	Cattle	Pollen	Boiled milk 1/100	Raw milk 1/100	Egg-yolk 1/100	Egg-white 1/100
−	−	−	−	−	+(+)	−	−	−	−	−	−
+	−	−	−	−	+(+)	+	−	−	−	−	+
−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	++	+(+)	+(+)	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−
+	−	−	−	−	+(+)	−	−	−	−	−	−
++	−	−	−	−	−	−	+	−	−	−	−
−	−	−	−	−	+	−	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	++	+(+)	+	−	+	+	−	−
−	−	−	−	−	+(+)	−	−	−	−	−	+(+)
−	−	−	−	−	−	−	−	++++	++++	++++	++++
−	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	−	+	+	−	−	+	−	−
++	−	−	−	−	+	+	−	−	−	−	−
+	−	−	−	+(+)	−	−	−	+	−	−	+(+)
+(+)	−	−	−	−	+(+)	+	−	−	−	−	−
−	−	−	−	+(+)	++	+(+)	−	−	−	−	−
−	−	−	−	−	−	−	−	−	−	++	−
−	−	−	−	+++	+	++	−	−	−	−	−
+	−	−	−	−	−	−	−	−	−	−	−
−	−	−	−	+(+)	−	−	−	−	−	−	−
−	−	−	−	−	−	−	+(+)	−	−	−	−
+	−	−	−	−	−	−	−	−	++	−	−
−	−	−	−	−	+(+)	+	−	−	−	−	−
−	−	−	−	−	−	−	−	−	+	−	−
−	−	−	+	−	++(+)	++	−	−	−	−	−
−	−	−	++(+)	++++	++++	+++	+	−	−	−	−
−	+	−	−	−	−	−	++	−	−	−	−
−	−	−	−	+(+)	++	−	−	−	−	−	−
++	−	+	−	−	++	−	−	−	+	+	+
−	−	−	−	−	++	+(+)	−	−	−	−	−
+(+)	−	−	−	−	+(+)	+	−	−	−	−	−
−	+(+)	+	−	+	+	+	−	−	−	+	+
−	−	−	−	−	+(+)	−	−	−	−	−	−
−	−	−	−	−	+(+)	+	−	−	−	−	−

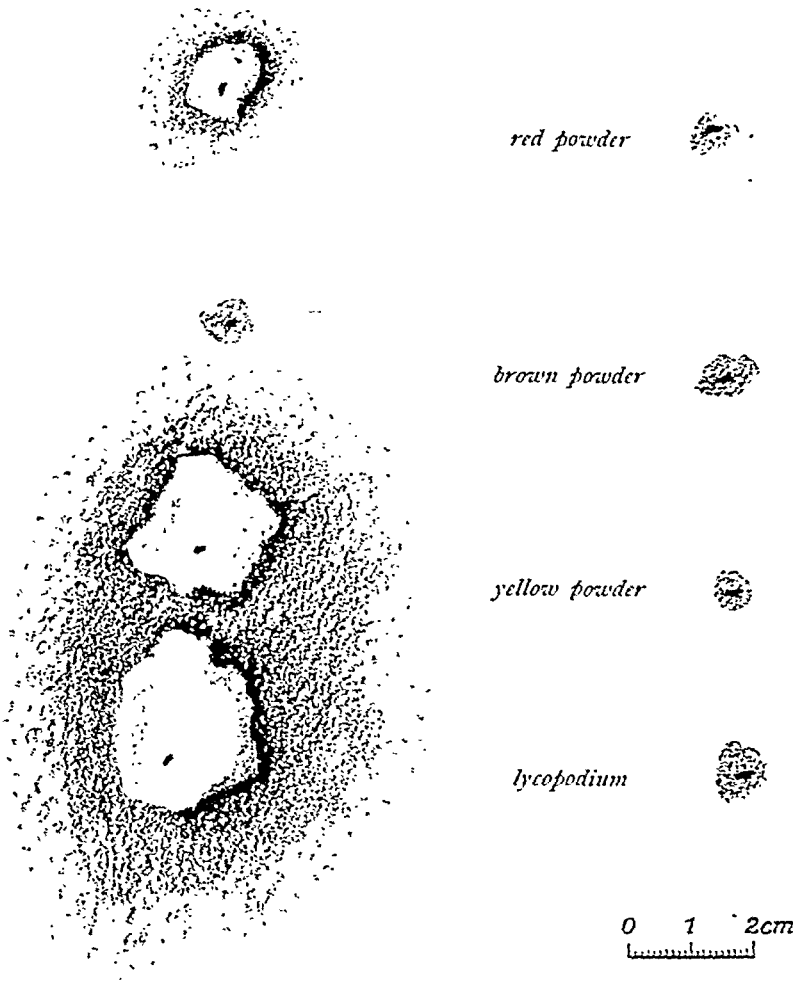

Fig. 1.

Attempt at passive homologous transfer with serum from a chorus singer at the opera[1]. She could appear in certain operas, but not in others. Result: The left vertical column shows the reactions when extracts of three different face-powders used at the theatre and of lycopodium were injected into *sensitized* areas of the skin; the right vertical column shows results of control injections into *unsensitized skin* of the same extracts in similar quantities. As will be seen there were strong reactions with lycopodium and yellow powder, weaker with red powder and negative with brown, results which agreed with the lycopodium content of the different powders and also with the patient's list of operas "tolerated" or "not tolerated."

[1] Complete description in Salén, Allergiestudien II, 1932.

The serological specificity and clinical significance of the positive skin reaction.

When the antigen analysis has been carried out lege artis and the precautions mentioned in the foregoing have been complied with, and the result has been one or more positive reactions, the question is to decide on two important points: are these reactions *serologically* specific? Are they *clinically* relevant?

First a few words about *serological specificity.* As to this I can be brief. Through extensive control experiments made by American authors, Walzer etc., by Pasteur Vallery-Radot and also by the present author, it must be regarded as an established fact that the positive reactions obtained by the direct skin test are usually reproducible in the experiment with passive homologous transfer, *thus involving the demonstration of specific antibodies or reagins in the blood of the allergic.* Our own numerous experiments in this connection, published in earlier papers,[1] do, however, call for a certain mount of reserve: if the reaction obtained in the direct test is fairly weak, with a titre of less than 1 : 100, the transfer test is not always positive, or the result is so doubtful that the positivity of the reaction cannot always be established with certainty. In other words, for some weaker reactions the serological specificity cannot be demonstrated with certainty. The bacterial reaction as a rule cannot be transferred passively, as already mentioned.

I shall give some illustrations

1) Transfer of a dust allergy (auto-dust extract).
2) „ „ „ Brazil-nut allergy *with peroral* excitation. (Fig. 2 A a. B).
3) „ „ „ lycopodium allergy. (Fig. 1).
4) „ „ „ chloramine allergy. (Fig. 3).

In this connection I would emphasize that it is not necessary to make the antigen analysis on the patient himself; it may be made on any other (healthy) person, provided that there is access to a small blood sample from the patient. This constitutes a

[1] Salén 2-6.

TABLE

Intracutaneous skin test of 24 school children

Name	Age	Heredity	Eosinophil %	Control	House dust	Mites	Bed-feathers	Mould	Human skin	Bacteria	Histamine 1/10000
S. R.	14	0	2	—	—	—	—	—	—	—	++
I. L. W.	14	+?	3	—	—	—	—	—	—	—	++
D. W.	10	0	1.5	—	—	—	—	—	—	—	++
G. N.	10	0	4.5	—	—	—	—	—	—	—	++(+)
N. N.	15	0	3	—	—	—	—	—	—	—	++
G. H.	13	0	4	—	—	—	—	—	—	—	++(+)
G. H.	11	0	3.5	—	—	—	—	—	—	—	++
V. M.	14	0	1	—	—	—	—	—	—	—	++(+)
L. G.	10	0	4.5	—	—	—	—	—	—	—	++(+)
E. A.	12	+?	1	—	—	—	—	—	—	—	++
E. B.	10	+?	4.5	—	—	—	—	—	—	+	++
H. F.	10	0	4	—	—	—	—	—	—	—	++
M. F.	13	0	4.5	—	—	—	—	—	—	—	++(+)
M. J.	10	+?	12	—	—	—	—	—	—	—	++
B. J.	12	+	7.5	—	—	—	—	—	—	—	+(+)
B. J.	10	0	4.5	—	—	—	—	—	—	—	++(+)
I. K.	14	0	3.5	—	—	—	—	—	—	—	++
H. K.	10	0	2	—	—	—	—	—	—	—	++(+)
S. O.	11	0	0	—	—	—	—	+	—	+(+)	++(+)
L. O.	11	0	1	—	—	—	—	—	—	—	++
I. L.	15	0	2	—	—	—	—	—	—	—	++
A. L.	15	0	3	—	—	—	—	—	—	—	++
H. L.	11	0	2	—	—	—	—	—	—	—	++(+)
S. K.	12	0	4.5	—	—	—	—	—	—	—	++

practical possibility which at times may prove to be of not inconsiderable value.[1]

The haptens which give a negative direct reaction also give

[1] An exploration of this possibility is of value especially in nervous individuals, small children, persons suffering from universal eczema, patients arriving from distant places and on whom specially prepared extracts outside the usual must be tried. Also as a control method for allergics with an abnormally high non specific skin sensitivity (see above).

4 without known allergy (18th December 1935).

Fish	Meat	Vegetables	Dog	Cat	Horse	Cattle	Pollen	Boiled milk 1/100	Raw milk 1/1000	Egg-yolk 1/1000	Egg-white 1/1000
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
—	—	—	—	—	—	—	—	—	—	—	—
(+)	slightly+	—	—	—	—	—	—	—	—	—	—
(+)	—	—	—	—	—	—	—	—	—	—	—
+	—	—	—	—	—	—	—	—	—	—	—
+	—	—	—	—	—	—	—	—	—	—	—
+	—	—	—	—	—	—	—	—	—	—	—
+(+)	—	—	—	—	—	—	—	—	—	—	—
++	—	—	—	—	—	—	—	—	—	—	—
+(+)	—	—	—	—	—	—	—	—	—	—	—
++(+)	—	—	—	—	—	—	—	—	—	—	—
++	—	—	—	—	—	—	—	—	—	—	—
++(-)	++	—	—	—	—	—	—	—	—	—	—
++(-)	—	—	—	—	—	—	—	—	—	—	—
++	—	—	—	—	—	—	—	—	—	—	—
++	—	—	—	—	—	—	—	—	—	—	—
++	—	—	—	—	—	—	—	—	—	—	—
+++	—	—	—	—	—	—	—	—	—	—	—
++	—	—	—	—	—	—	—	—	—	—	—

a negative transfer test. In conclusion I shall show a transfer test of a chloramine allergy.

I shall revert later to the clinical evaluation of the weak direct reactions.

I shall now proceed to the other main question: *the clinical evaluation of the results of the skin test.* Fairly comprehensive comparative experimental series between non-allergics and allergics have been published in various quarters (American authors,

W. Berger a. o.). I reproduce such a series which Hulting, Nordenfors and I worked out together in connection with our work in the School Home for Asthmatic Children at Stockholm. These tables 3-4 speak for themselves, and the only comment I would make is that a young girl who was placed in the healthy group and produced a number of fairly strong positive reactions, on closer inquiry was found to be suffering from a periodical urticaria and thus rightly should be transferred to the allergic group. I may add that this material covers children under 12 years of age. Already here I shall draw attention to two details: firstly the rare occurrence of fairly strong positive reactions with bacterial allergens, this in contrast to what is the case in older allergics; secondly, the strong egg reaction in the case of a boy to whom I shall revert later on.

Now, what does a positive reaction prove from a clinical point of view? To what extent should it—or could it—be used as a basis for clinical measures? In answering these questions a number of different viewpoints must be taken into consideration.

Let us therefore start the discussion with the question of the possible clinical importance of the weakly positive reactions (with a titre of or about 1 : 100). In an earlier work, published in 1936, we have expressed the opinion that from a clinical point of view such reactions are rarely or never of any *actual* significance. Sometimes the same holds good even of reactions with a titre of up to 1 : 1000. In this connection it is however especially important to point out the amount of reserve which the word *actual* embodies. Such a weak or relatively weak reaction may constitute a reminiscence of a previously strong reaction which may have been of a clinical significance or perhaps quite dominant. But *it may also be an expression of a quality of hypersensitiveness which is continually growing* and which later on may become clinically significant, even dominant. I shall illustrate this with a drastic case:

In 1936 we made an antigen analysis on an eleven year old boy in whom the main symptom was that of asthma. The result of the antigen analysis seemed to reveal that it was a case of a clear *dust* allergy. Some weak reactions of a different kind were certainly obtained in addition, i.a. a $\pm$ to $+$

reaction with horse antigen. Neither through the questionnaire nor by separate questioning was evidence of a clinical horse allergy discovered.

About ten years later the patient noticed that asthma symptoms appeared when he was in a stable or came near to horses. From later questioning it appeared that he had not had horse serum injections during the intervening years. He was now helping in a bacteriological laboratory, where it chanced that a horse extract was being prepared. When about to leave one night he caught sight of the bottle of extract and became interested to know whether he would react positively to a skin test with it. He injected a small quantity of the extract intracutaneously into himself. After a few minutes; Status asthmaticus, shock, death!

I regret, however, that time does not allow me to relate further examples to show how a quite weak allergen reaction in a test, which then in all probability is without any clinical significance, at a later test may have grown in strength and then have acquired clinical significance: this circumstance constitutes a proof that a fresh antigen analysis should be made in cases where allergic symptoms reappear. This is a particularly important rule i. a. especially in the question of judging and appraising the results of the treatment or the durability of any improvement thereby obtained.[1]

In a work on latent allergy, Juhlin-Dannfelt and I have been able to determine the rather frequent occurrence of a clinically latent allergy within certain occupational groups and for certain "occupational" allergens. We have thereby been able to verify the appearance not only of weak or moderate allergen reactions, but at times strong ones. The serological specificity of these reactions could even be stregthened through positive transfer tests.

It seems clear that these verifications apply not only to the qualities of hypersensitiveness thereby tested, but also to those of a different nature such as dust, pollen etc. In this connection we pointed out that in similar cases it is sometimes impossible to decide whether the reaction obtained has had a previous clinical significance, whether it may have in the future or—as a third possibility—whether it has been or will become an expression of a *latent* allergy.

[1] If the circumstances are not thoroughly investigated from this point of view in every case, faulty conclusions may easily be drawn.

If this reasoning is accepted, it should follow that in the antigen analysis we are sometimes bound to encounter strong, even very strong reactions which lack actual clinical significance, but among which perhaps there was one which did have that significance previously, and another that may acquire it later. If this is so, these possibilities are of the greatest importance to the clinical evaluation of the result of the antigen analysis and will require special attention. That such possibilities—though they are rare in my experience—do actually exist I shall illustrate by the following example:

I spoke of the case of the small boy who formed part of the School Home material and who reacted strongly to eggs (Table 3). When questioned, his mother explained that the boy ate eggs every day without showing signs of any trouble, but that *before* the onset of his *dust asthma* he had been suffering from a severe itching rash for several years (evidently Prurigo Besnier). The mother now stated that at that time he was so hypersensitive to eggs that the ingestion of the smallest quantity brought about a strong exacerbation of his eczema. At the time of the onset of the asthma some years ago the eczema disappeared and—as already mentioned—he had been able to eat eggs without discomfort. The antibody content in the blood of this little patient was, however, still so high that the test of passive homologous transfer gave a reaction by *peroral* administration of egg to the experimental subject.[1]

During his stay in the School Home we were able to verify the mother's statement that the boy could now eat eggs without trouble.

This case brings us near the so-called *dissociated allergy*. An example:

A young woman consulted us for allergic rhinitis. The antigen analysis showed rather strong reactions to dog and dust extracts. Besides the usual measures for her dust allergy we prescribed avoiding contact with dogs. However, she had a small terrier which she did not like to part with and, consequently, decided to make some tests herself. She put her nose into the dog's coat and kept it there for quite a while: no nasal catarrh. She soaked

[1] This case, moreover, was one of those (see also Fig. 2 B) where we were able to excite the reaction perorally also *a second time* (*without a fresh sensitization in the interval*). Tests with peroral excitation were first made by Walzer, shortly afterwards by us in a case of paranut (Brazil) allergy. Since then we have at hand about 30 such highly active serums.

a piece of cotton wool in the dog's saliva and put it into her nose: no catarrh. But *every time she stroked the dog's coat a strong urticarial reaction appeared on her hand and upwards on her forearm.*

The appearance of such dissociated allergy must of course be given full consideration in the clinical evaluation of the results of the analysis. It is of frequent occurrence especially in the combination asthma + Prurigo Besnier.

A second case, of which a brief account shall be given here, indicates the presence of another possibility:

A middle-aged woman produced a rather strong lycopodium reaction to the skin test. When questioned she said that she had not noticed any inconvenience when going to the opera etc. nor in connection with a shampoo. A cautious exposure test gave a negative result. She consulted us for allergic rhinitis and said she had never had any asthma symptoms. During the treatment she was relieved of trouble, and lived content and happy for the next six months. Then I was called to her in haste and found her in a serious Status asthmaticus. After careful questioning it appeared that the attack had come like a bolt from the blue while she was having a dry shampoo. According to the information received later on from the manufacturer the shampoo in question contained *90 to 95 per cent. lycopodium.*

As a last important question in this connection I would ask: Is it possible that a patient can give a negative reaction to an allergen,[1] which anamnestically-clinically, or by exposure and elimination experiments can be proved with certainty to be of causal importance? Reports in the literature vary a good deal, are often vague and it is seldom authenticated that due consideration has been given to all sources of error in the question of the reliability of the extract etc. As far as our experience goes, I can only remember one or two cases where it seems that this possibility could not definitely be excluded. That it is very difficult to obtain fully valid allergen extracts in certain cases (for instance from strawberries etc.) will be well known. The possibility that certain (bacterial, nutritive) allergens in their action resemble the haptens, or that their antigenic function arises out of the break-down products of the digestion process, has already been hinted at in

[1] Complete inhalation allergen.

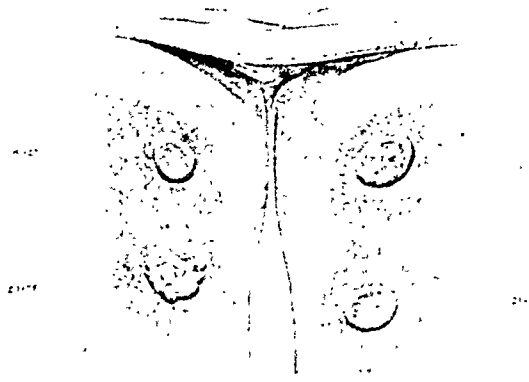

Fig. 2 A.

Of serum from a person markedly hypersensitive to Brazil nuts 0.1 ml. was injected intracutaneously at four places on the anteromedial aspect of the upper thigh (Feb. 5th). After 24 hours (the injection sites then showing no reaction whatever) the patient ate a few Brazil nuts. After 5 to 7 minutes came the picture here illustrated. The same experiment on about 100 healthy persons had the same result, even when the Brazil nuts were administered endonasally or perrectally. The response to a direct test on the patient was a dangerous anaphylactic shock[1]. The serum was still highly potent in 1947, after being stored nearly 15 years!

the above. In any case I venture to maintain that this possibility relatively seldom occurs.

If we sum up part of the above data it will appear that the serological specificity cannot always be verified by positive transfer tests where it is a question of *weak* positive reactions, and that such reactions as a rule do not have *actual* clinical significance. Under such circumstances it would seem justifiable, when making a statistical analysis of an allergy material, to determine, by means of careful titrations of the strength of the hypersensitiviness, the frequency of such *stronger* skin reactions as will with fairly great probability be of clinical significance. On account of what we have said above, we have found it convenient to shift the titre limit to 1 : 1000 or more. However, that even strong reactions can be clinically irrelevant we have earlier proved (p. 150).

[1] Complete description in Salén, Allergistudien II (1932).

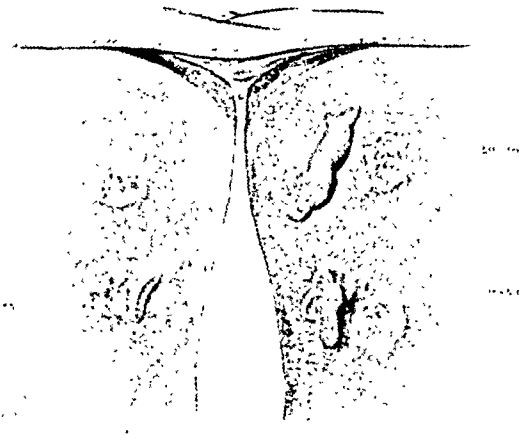

Fig. 2 B.

On Feb. 9th the experimental subject employed in Fig. 2 A—*without fresh sensitization!*—again ate some Brazil nuts. Result: after about 10 minutes the picture reproduced. As will be seen, the reaction now is almost completely absent from the central sites of Feb. 6th and instead appears mostly in a position medially proximal to them (in the direction of the regionary lymph-gland centres). In this case excitation was still possible about six weeks after sensitization.

How often are positive allergen reactions obtained in skin tests and how often are they of clinical value and significance?

In seeking answers to these questions in the available literature it will be found that the reports differ to a great extent. The causes of such a marked lack of uniformity are certainly many and varied. A number of these causes have been described or indicated in the above. The choice of extracts, their mode of preparation, their standardizing, the technique of the skin test itself, the estimation and evaluation of the results, all these are variable factors and doubtless of variable significance in different countries, different hospitals and to different research workers. All this partly explains the kaleidoscopic picture which impresses itself on the mind in its search through the literature for an answer to these questions. Here an attempt at a thorough "standardizing" would undoubtedly more than ever be well founded and desirable.

In the above we have dwelt at some length on the precau-

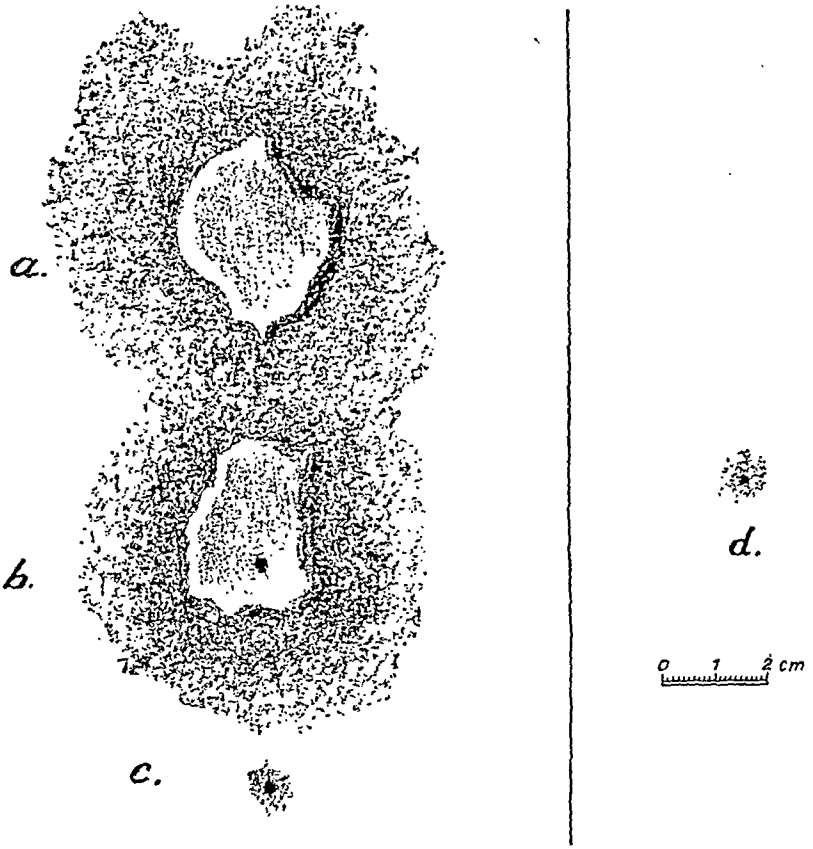

Fig. 3.

0.1 ml. serum from a stricking case of chloramine allergy in a veterinarian was injected (November 30th) intracutaneously in two places on the left forearms of a healthy person. On December 5th the injection sites could not be recognized with certainty, though two, *c* and *b*, were suspected. Assuming that these were the sensitized skin areas we injected at *c* 0.1 ml. 0.5 % chloramine solution (in physiological saline), *b* 0.5 ml. saline only. As control 0.1 ml. 0.5 % chloramine solution was injected in the right forearm. Within 5 minutes there was intense itching at the sites *a* and *b*, redness, eruption of small blisters which gradually merged into large plaques surrounded by erythema. After about 20 minutes the picture shown.

tions which in our opinion must necessarily be taken into consideration if, from a clinical point of view, the functional antigen analysis is to attain the value and importance it undoubtedly deserves. The importance of *biological* standardization must be specially pointed out. This means, however, that the extracts used in diagnostics must have been tried on a fairly large number of healthy, non-allergic persons and yielded clearly negative reactions. The extract is only useful in the dilution which gives this result. As mentioned before, we have tried to make the situation more secure by using a further dilution: 1 in 10 of the dilution thus obtained. In passing it must be emphasized that often it is not at all difficult to prepare for example a dust extract so concentrated that it produces strongly positive reactions in the majority of all non-allergic subjects. Indeed, if one is unlucky the test may even result in a severe, deeply necrotic, process!

But: other factors do certainly also play a decisive part as a causal factor in the aforesaid marked difference. As Salén and Arner have illustrated statistically in a lecture on aspirin allergy at this Congress, negative skin spectra occur (through the lack of suitable bacterial allergen extract?) in about two-thirds of all cases of aspirin hypersensitiveness, somewhat less frequently in the other great infection-complicated asthma group. With a few exceptions the whole of the "hapten" allergy group produces negative skin reactions. In marked contrast to this we find, according to the same authors, clearly positive spectra in about 85 per cent. of the less complicated, more purely "exogenous" cases of allergy.

It will appear from the above that, according to the nature and composition of the material, the percentage of cases with positive skin spectra is bound to vary greatly, according to statistics between just over 30 and almost 90 per cent. It is therefore obvious, too, that if cases of allergy are sifted by experts, for example at a policlinic for allergics, and only the sifted cases are hospitalized, this sifting may, all according to the criteria employed, result in greatly varying percentages of positive skin tests in the clinical material.

Against this background the statements must be seen and

estimated, and only then will it be possible to understand and explain the marked differences they contain.

The asthma clientele, which earlier constituted a heavy burden on our hospital wards for internal diseases, consisted, as is well known, mainly of old, secondary- and infection-complicated, frequently more or less disabling cases of asthma. If with such a material one should endeavour with interest to go in for time-consuming allergy investigations, antigen analysis etc. it is a priori clear that the vague and, to a great extent, negative character of the results would cool down even the most red-hot interest very quickly; so much the more as the results would occasion anti-allergic measures, treatment etc. in only few cases.

In sharp contrast is of course the group of non-complicated, "exogenous" cases of asthma where the skin test often gives clear evidence of the causal factors and where the anti-allergic measures based thereon, be they specific or non-specific, or both, imply and signify a change in the prognosis to the better, one which for juvenile asthma approaches 100 per cent! When it is considered that a great number of the chronically infection-complicated, more or less disabled cases have grown from this group, so that the primary allergy has favoured the onset of secondary infection complications and created a circulus vitiosus, it must stand out clearly as the fundamental object of modern allergy research work and treatment to get hold of the allergy cases at the earliest possible stage for examination and treatment. Only then is there a hope that the group secondary infection-complicated and disabling asthma cases may be eliminated *gradually*. This goal must now and always be the main object of modern allergy research. In all probability the road to it will be long and thorny.

Feinberg seems to give a moderate figure for the percentage of skin-positive allergic cases when he places it at 50-60. Of the nature and condition of the material he gives no details, however. With material from our clinic at the "Sabbatsberg Sjukhus"—a municipal hospital for promiscuous internal cases—Juhlin-Dannfelt has made a comparison, comprising 718 cases from the years 1941-43, of which he found that the skin-positive cases amounted to 58.8 per cent. The corresponding figure for the cases which

produced one or more *stronger* skin reactions (titre at least 1 : 1000) is 51.5 per cent. for this material. In this, however, it was not possible to consider bacterial allergen reaction, as no suitable extract was available during the period mentioned. As the greater part of the Sabbatsberg material consisted of old, chronic asthma cases, it must be supposed that the percentage would be considerably higher for an all-round material. This supposition is likewise supported by the fact that the corresponding figures for cases in the allergological clinic of the Söder Hospital (Södersjukhuset) in recent years were about 10 to 15 per cent. higher, and E. Bruun finds (1945) of a material from the Rigshospitalet at Copenhagen only 5 per cent. that did not give a positive reaction.

As the establishing of special clinics for allergic cases presupposes that these clinics should be reserved for a *selected* material, first and foremost consisting of relatively fresh cases of allergy in which the allergy investigation gives fairly clear information as to the necessary anti-allergic treatment and gives reason to hope for the effectivity of such treatment, we have thought it of interest to analyse statistically the material from the Privata Sjukhemmet at Stockholm. This hospital has from the beginning (1934) been dust-cleared—in this respect it served as a model for the School Home for Asthmatic Children at Stockholm.[1] The symptomatic treatment has on the whole been the same as that of the aforesaid Sabbatsberg material, with the exception that the eucalyptus-tar treatment has been employed as routine treatment at the Privata Sjukhemmet. Only selected cases were admitted. The number of asthma patients who consulted me privately from 1941-46 was about 1,300. Out of this number 809 (62 %) were treated in the Privata Sjukhemmet, about 500 were out-door patients. I regret to say that I have not been able to analyse this latter group statistically, but it includes in the main *two* categories of cases: one, and decidedly the larger, comprising almost exclusively non-complicated *dust* allergies, which have been mastered through a dust clearance in part of the dwelling and a non-specific anti-allergic injection treatment (usually allergol treatment). The other main

[1] See Salén, Hulting and Nordenfors.

group consists of cases with chronic infection complications, which were directed to the local hospital for infection clearance or treatment. In the Sabbatsberg hospital the patients were mainly from Stockholm, in the Privata Sjukhemmet mainly from the provinces. A number of dust allergies with a titre of more than 1000 belong in all probability to the former group, a number of cases with dust hypersensitiveness to the latter. The material, 809 cases, consists in the main of asthma cases for which the antigen analysis gave fairly clear directives as to special anti-allergic measures. To this comes, however, a number of cases with protracted, sometimes purulent bronchial infections which were considered to require inhalation treatment. This explains the high percentage of skin-positive cases in the allergy group described above (table 5) and also the high percentage with a titre of at least 1000 (76.8 per cent.). It must be added, however, that the majority of these 809 cases had severe clinical symptoms and most of them had previously been treated in other hospitals without a lasting result. *This material is perhaps of the greatest interest, from the point of view that it proves how the primary investigation and the antigen analysis make it possible to select such cases which a priori may be supposed to be accessible for special anti-allergic measures, and the treatment of which in a special hospital for allergic cases is therefore especially well founded.* As already stated this material, however, also includes a number of infection-complicated cases, though most of them could be cured of the infection while in the hospital.

In 76.8 per cent. of these sifted cases the titre was not less than 1000, that is, they reached the limit which, for reasons mentioned above, I considered it expedient to draw in order to ensure an approximately reliable elimination of doubtful or clinically non-actual skin reactions. In 74 per cent. a specific desensitization was carried out, in the majority of the cases against two or more qualities of hypersensitiveness. In a great number of cases the titre rose to 100,000, in quite a number to 1,000,000 and in a few to ten millions. A protracted state of infection, with durable increases of the sedimentation rate, occurred in some cases. We shall go further into this matter in connection with an account

of the cases of aspirin hypersensitiveness. In spite of the fact that desensitization was carried out with the greatest care, we found that clinical shock symptoms appeared in no fewer than 212 of the 597 (35·5 %) specifically desensitized patients.

As will appear from Table 6, *juvenile asthma* stands in a class by itself among the asthma cases. *As a rule* the causal factors are clearly revealed by the antigen analysis, and, *as a rule,* the anti-allergic treatment based thereon gives a satisfactory result. Even if a not unimportant number of infection-complicated cases are also found in this group, it holds good of such cases almost without exception that they can be eliminated without difficulty (infected adenoid, tonsillitis etc.). The change in the prognosis in a favourable direction is so marked here[1] that the cases where the results are not satisfactory belong to the relatively rare exceptions. Such exceptions are cases where secondary anatomic changes of a significant order have already taken place. *The group juvenile asthma is so far of special, fundamental interest as, widely speaking, it forms proof of the extremely favourable result which may be obtained with modern allergy treatment of relatively uncomplicated, early cases of allergy.*

If thus the frequency of the positive skin reaction varies in the different categories of asthma cases, it is of special importance to establish the fact *that it is impossible in advance to judge* the outcome of a case in the antigen analysis. *Every single asthma case should therefore be subjected to such analysis.*

In this connection I would ask the following question: is a negative skin test of an individual who historically and clinically is definitely allergic devoid of all value? First and foremost I shall refer to what has been said in the above about the importance of supplementing in every case the series of extracts with any special extract that *may seem likely* in the particular case. If this condition has been complied with and the skin spectrum remains negative, and if historical-clinical data indicate that there is a clear allergy, then in our opinion there is the greatest probability that we have to do with an *endogenous* factor, usual-

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[1] See Salén (10), and E. Bruun, Nord. Med. 28, 2581, 1945.

TABLE 5

Summary of 809 cases of allergy treated at the Privata Sjukhemmet 1941-46, including 794 cases of bronchial asthma. In 14 of these 794 cases there was also allergic eczema.—This material includes 183 children under 12 years.— The number of cases of aspirin hypersensitiveness was 42 (5.2 %). All the 183 children tolerated aspirin.

A. Number of positive reactions.[1]

Total No.	0	1	2	3	4	5	6	7	8	9—11	Total skin posit.	No. of cases with one or more qualities of hypersen. with titre 1:1000 or higher
809	48	154	188	158	110	70	42	15	15	9	761	618
%	6	19.2	23.3	19.7	13.6	8.9	5.3	1.9	1.9	1.2	94	76.5

[1] Between 60 and 70 % of the skin-positive cases reacted positively to one or more of the dust extracts used in the tests.

B. Number of qualities of hypersensitiveness for which specific desensitization was carried out.[1]

	0	1	2	3	4	5	6	7	8—10	Total cases desensitized	No. of cases which during desens. reacted w. clinical allergic symptoms
No. of cases	212	188	150	120	70	22	25	11	11	597	212
%	26	23	19	15	8.5	2.5	3	1.5	1.5	74	35 5 (of those desensit.)

[1] The specific desensitization was carried out as a rapid cure, usually with 5 to 7 injections per day.

TABLE 6

Summary of 183 juvenile asthma patients under 12 years, treated at the Privata Sjukhemmet 1941-46.

A. Number of positive skin reactions.

Nr.	0	1	2	3	4	5	6	7	8	9	10	11	Total skin positive cases	Titre 1:1000 or higher
183	3	29	46	31	28	23	11	3	5	2	1	1	180	163
%	1.6	15.9	82.5										98.4	89.1

B. Number of qualities of hypersensitiveness for which specific desensitization was carried out.[1]

	0	1	2	3	4	5	6	7	8	9	10	Number desens.	Clinical reactions
	29	43	42	25	22	5	9	3	2	2	1	154	68
%	16	24	60									84.3	44.2 (or the No. spec. desens.)

[1] Desensitization could not be completed (at the usual highest dose) in only 3 cases on account of severe clinical allergic reactions.

ly a (chronic) infection complication as the main causal foundation. We must then resort to the whole arsenal of clinical examination to reveal (or exclude) the presence of such a factor. If an effective infection clearance can be carried out, the allergy diagnosis is often strengthened ex juvantibus.—*If there is aspirin hypersensitiveness, this forms a near enough proof of the allergic character of the disease and of the presence of a chronic infection process* (Salén and Arner).

Value of Exposure-, Provocation- and Elimination-Tests.

In conclusion a few words about the value of the exposure, provocation and elimination tests to the evaluation of the skin test. In cases of more severe allergy the questionnaire frequently gives information which makes such tests superfluous, and indeed their performance often involves the risk of severe shock. In cases of less pronounced hypersensitiveness, positive results are not always obtained by the exposure tests, probably due partly to the variations of the degree of the manifest allergy which seems to be present. Our experience from the tests made on the children in the School Home, i.e. mainly cases of severe dust allergy, has taught us that, after some time away from the dust milieu, the children often have no trouble the first night of their return to their homes, but the second or the third. This experience seems to us to be of importance, worth while taking into consideration in a discussion on the reliability of the exposure test. At the present moment it is hardly possible to estimate to what extent an additive effect of several simultaneous qualities of hypersensitiveness, each one perhaps not strong enough to produce clinical reaction, plays a part and is of importance in this connection. Vaughan, however, accepts the existence of this possibility as a fact. That in the case of nutritive allergy the exposure or elimination test is often very useful, should be taken for granted. A negative provocation test should not be credited with decisive significance.[1] If repeated, such tests will naturally gain in value.

[1] If one should venture clinically to resort to intravenous allergen injec-

In the carrying out of specific desensitization one will find, as will be seen from the table, that shock symptoms are relatively frequent. The results ex juvantibus should be given a high value as evidence of this.

SUMMARY

Concerning historical data and synopsis, in connection with the important question of the preparation, choice and standardizing of the allergen extract, the technique and precautions in the carrying out of the antigen analysis and the principles for its evaluation etc., I shall refer to what is stated in the above.

It is of importance to keep in mind that the antigen analysis is an important diagnostic *adjuvant* in allergic diseases and of significance to their clinical judgment and the therapeutic procedure *where an ordinary internal-medical examination in every allergy case constitutes an indispensable complement.*

The *serological specificity* of a fairly strong positive skin reaction may as a rule be checked and verified by means of experiments with passive homologous transfer, its clinical significance often through exposure, provocation and elimination tests as well as ex juvantibus. For weaker positive reactions (with titre of about 1 : 100) the serologous specificity often cannot—or not with certainty—be proved through the control test mentioned above. It is of importance to underline the occurrence of *latent* and *dissociated* allergy.

Further it is of importance to remember the existence of the quantitatively significant groups *endogenous* (first and foremost bacterial-toxic) and *hapten* allergy, in which as a rule positive skin tests are not obtained, nor do transfer tests give positive results. *From this it follows that in a considerable number of allergy cases a positive result of tests with exogenous allergens cannot be expected. All allergy cases should, however, be subjected to the antigen analysis,* partly because one is rarely or never able

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tion, the result in a high percentage of cases would doubtless be instructive, but—macabre.

to judge beforehand whether an exogenous allergy is exerting an influence and is of *contributory* causal importance, *partly because a negative skin spectrum in an historically-clinically determined allergy argues the presence of an endogenous causal factor, and, if so, usually a bacterial-toxic process.*

It is furthermore of importance to remember how the whole conception of "allergic diseases" from the beginning was built mainly on experience gained from the antigen analysis. Further, how the use of this analysis and the anti-allergic measures founded upon it change the prognosis to a high degree in a favourable direction, *especially in the non-complicated allergy cases and quite specially in the cases of juvenile asthma.* So far have we come from the time when—slaves of medicamentation—the majority of cases were regarded as chronic, incurable diseases, not infrequently terminating in a chronic, disabling course. *Seen from the point of view of the modern allergy doctrine the alpha and omega must be to get cases of allergy for examination and treatment as early as possible. Only then shall we be justified in our hopes that the number of chronic, disabled cases, which now constitute such a heavy burden on our hospitals, will be gradually decimated.*

The antigen analysis is a subtle examination method, involving no small risk and in procedure, judgment and clinical evaluation requiring wide experience in the domain of allergology. Like the majority of other clinical diagnostic methods, it is encumbered by a number of sources of error which demand the strict observance of certain precautions mentioned in the above. The preparation, standardizing and choice of extracts are subtle processes, requiring experience and special resources, which usually only the specialist has at his disposal. Many circumstances therefore argue strongly that for the time being the functional antigen analysis should only be handled by one who has acquired profound experience. A departure from this principle may easily jeopardize the reliability and value of this diagnostic method.

The significance of the antigen analysis must be specially emphasized when a choice of occupation is involved.

Subject to these conditions the antigen analysis must be

acknowledged to be of *fundamental* importance from a diagnostic and therapeutic point of view. It is of the greatest importance that all cases suspected of allergy should be submitted to the analysis as early as possible.

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Discussion: see page 176.

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THE DIAGNOSTIC AND CLINICAL IMPORTANCE OF THE SKIN TEST

SUPPLEMENTARY REMARKS TO DR. SALÉN'S PAPER

By

P. BONNEVIE,
Copenhagen.

The tests of the skin reactivity employed for diagnostic purposes are of many different kinds, even when we include only those tests which are aimed at disclosing a special reaction to a substance introduced into the skin at a single spot. On the other hand, they also have much in common.

If we limit the tests to procedures from which a *special* reaction will appear, we leave out those for instance with histamine and morphine, which are of diagnostic value in deciding partly the reactivity of the skin capillaries, partly the arterial supply of blood to the skin, and which merely display quantitative differences in the normal vascular reaction, differences which, by the way, we must often reckon with in the strength of the special reactions. But what is required to be demonstrated by the allergic skin tests is principally a *qualitatively altered* reactivity, that is to say a reaction of another kind than the normal, or a reaction of a particular kind where normally there is no reaction.

This is not contradicted by the fact that allergic skin tests can be carried out quantitatively (so-called allergometry). One either measures the strength of the characteristic reaction to a certain amount of the test substance or—which is nearly always better—one finds the least quantity of the test substance capable of exciting the characteristic reaction.

The difficulties met with in allergic skin diagnostics lie most of all in separating the diagnostically useful special reactions from the diagnostically misleading simple reactions of varying strengths.

It must be pointed out here before going any further that

the possibilities of the skin's giving morphologically different reactions of an inflammatory character (in the widest sense of the term) to locally administered substances foreign to the organism is rather limited. As allergic skin diagnostics in principle endeavour to avoid the use of substances with a toxic necrotizing effect, at any rate in the quantities employed, it will suffice here to consider the following three reactions:

1) the rapidly appearing *capillary reaction* (triple response) with urticarial papules,

2) the more slowly appearing *stroma reaction* of an inflammatory character, and

3) the also more slowly developed *epidermis reaction* of the eczematous type.

The urticarial capillary reaction is the morphologically special reaction in the scratch and injection tests commonly employed in allergology; *Rokstad* therefore justly prefers to call them *urtica tests.*

The inflammatory skin reaction is the special reaction looked for in the scratch and injection tests used in allergic skin diagnostics in infectious diseases, *Pirquet*'s and *Mantoux*'s tuberculin tests being those most frequently applied. The inflammatory reaction can also be excited by epicutaneous application of the substance, for example *Moro*'s inunction of tuberculin, tests with plasters of which the adhesive is mixed with tuberculin, or by patch tests, i.e. tuberculin absorbed into a small piece of cloth or filter paper and placed upon normal skin, held in position by adhesive tape with a layer of impermeable material, for instance cellophane, between. Inflammatory skin reaction is also obtained by intracutaneous injection of substances which excite eczematous epidermis reaction and are usually accompanied by that reaction; there is much to indicate that in epidermal eczema reactivity there is often, though not always, a separate inflammatory reactivity of the corium. Naturally it is difficult to decide whether the changes in the corium of a simple inflammatory nature accompanying the epidermis reaction, are merely secondary, accompanying phenomena, as an inflammatory change in the epidermis must always be accompanied by vascular changes.

Salén, E. B.: Of the contributions to the discussion I think Professor Sonne's was the most important in principle. As at the Northern Congress of Internal Medicine in Helsingfors about ten years ago, Professor Sonne today again directed his criticism against the exaggerations which modern allergy teachings may nourish in their bosom. Professor Sonne seems inclined to accord psychogenic causal factors a greater role than allergy enthusiasts do. It need hardly be pointed out how in particular paroxysmal asthma must be calculated to produce nervous perturbation in a patient. Unawares, fitfully, he is struck at any time by perhaps a severe attack like a bolt from the blue. From being in the best of health he is suddenly brought to a state of severe illness. Such a patient often has stubborn difficulty at night which deprives him of sleep. All this will certainly help to *bring on* nervous emotion, even in the case of a person whose nerves are otherwise quite in order.

One who is occupied with these diseases has opportunities almost daily of observing how even slight allergic manifestations *secondarily* are capable of involving nervous symptoms. To give an example: a lad attending school in the exacting third form had always been exemplary and never neglected his studies; he began to be "slipshod", seemed "nonchalant", his school work was poor; he gave the teachers and his parents the impression of being nervous, irritable, not like he was before. The only morbid or abnormal symptom noticed was a fairly heavy, trouble-some rhinitis of the *morning* type. It was said to have set in with a cold some months before. When examined he presented a normal sedimentation rate, moderate blood eosinophilia. The antigen analysis resulted in fairly strong reactions with dust and bacterial extract. Nasological examination revealed an ethmoidi-tis, which was treated operatively. Partial dust desensitization was carried out and the patient was given an allergol cure. After two or three weeks he was free of his symptoms, had no trouble. The psychic change, the nervous symptoms, had disappeared. To his teachers and relatives he is now the same ambitious youth as before.

At the Helsingfors congress I cited as an instructive example the case of hay-fever patients. If one has the chance of observing a number of these *out* of the season, the impression gained is that usually they are individuals without psychic blemish or

ammonium persulphate, used in Denmark some years ago as a socalled flour-improver. The intracutaneous injections were 1: Am. persulphate 0.5 ‰ in physiological salt solution, 2: control (glucose), 3: wheat extract, and 4: wheat extract + am. persulphate. Eczema test P: am. persulphate 5 % in water, B: potassium bromate (another flour-improver).

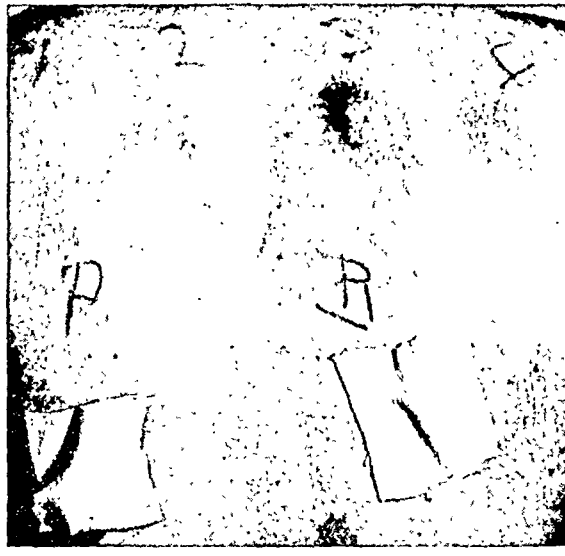

Fig. 1.

Fig. 1 shows the urticarial reaction to wheat, Fig. 2 inflammatory delayed reactions to and positive eczema test with am. persulphate. The patient, a baker, had had eczema due to persulphate and suffered from baker's coryza due to wheat flour.

Fig. 3 shows the urticarial reaction to am. persulphate (not wheat), Fig. 4 inflammatory delayed reactions and positive eczema test with am. persulphate. The baker had had persulphate eczema and suffered from coryza due to flour containing persulphate, whereas he was well while working with pure flour.

Time will not permit of going into the many pitfalls of the eczema test and its diagnostic significance. I shall merely mention a few matters of general interest in allergic skin diagnostics.

The reaction to an eczema test is positive only if it discloses one of the characteristics of the eczematous affection to be diagnosed: *morphologically* the typical eczema papules and vesicles, *developmentally* a latent period, which is of particular significance if the test results merely in a more or less infiltrated erythema, or *by the "positivity"* that the substance is not known to cause

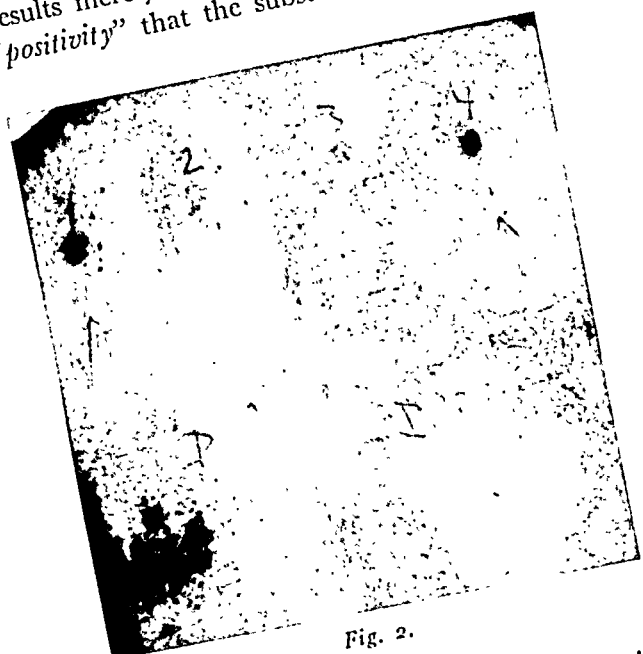

Fig. 2.

any form of toxic reaction on normal persons, this being of special importance if there is only a faint redness reaction without nakedeye changes in the epidermis.—Applied to the skin tests generally employed in allergology, it must correspondingly be required that the test gives *an active, urticarial reaction with its characteristic morphological features*, not merely an extension of the wheal caused by the injection (owing to the distribution of the injected fluid or its attraction of lymphatic fluid). Therefore the smaller the quantity of fluid injected, the better, preferably only 1/100 ml. Furthermore, the urticarial reaction must appear *within a short time*, corresponding to the period of latency in the case of a massive excitation of the mucosa of the nose, bronchi or

intestines. And finally, the injected test substance *must not contain substances toxic to the capillaries* to excite the urticarial reaction —perhaps in varying strengths in the various individuals.

The reaction of an eczema test is only negative if the test is made with a concentration of the test substance lying *just below*

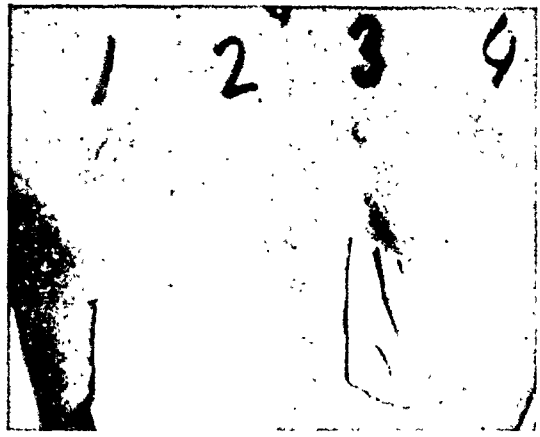

Fig. 3.

the concentration that is capable of producing a toxic, corrosive reaction; the point is that in several cases the allergy is so weak that, under the conditions of the test, low concentrations do not bring substance enough to excite the reaction; in fact, in certain cases, in order to demonstrate an incipient allergic sensitization it may be necessary to go up to corrosive concentrations and cause reactions that are toxic and eczematous in combination, and, for their analysis, require a special method of application (*Rokstad*'s terpene tests by the adhesive chamber method).—Applied to the ordinary urtica tests, this means that one cannot decide *generally* to test only up to a certain concentration, for example 1 per mille (the concentration of the allergen obtained by shaking 1000 ml. fluid with 1 g. of the initial material), or to use as a criterion of positivity that the reaction can be excited by a certain concentration in all cases, for instance 1 : 10,000. These are only quantitative abstractions, whereas the investigator should think in terms of quality, morphology and biology.

Reading an eczema test is uncertain, or at any rate requires great care and long experience, if the adhesive plaster also causes an eczematous reaction. It corresponds to the compromising of the urtica test by dermographic capillary lability through trauma caused quite mechanically by the scratch or the injection.

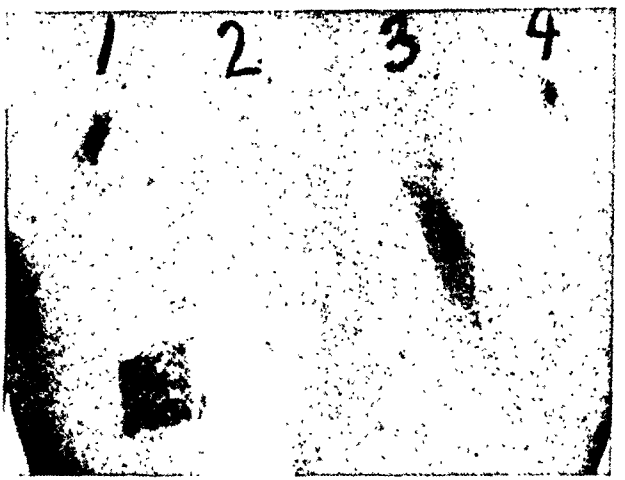

Fig. 4.

An eczema test may give *an unspecific, but morphologically typical* eczematous reaction in conjunction with a simultaneous, genuine, positive eczema test or already existing acute eruption or exacerbation of the present eczema. The action of the test *provokes unspecifically at the test site a "metastatic" localization* of the experimental or the natural eczema. The very small quantity of antigen absorbed and circulating in the blood apparently "localizes itself" at a spot where a slight capillary trauma, which otherwise would not have become manifest, forms a "locus minoris resistentiae." This means that one cannot venture to decide whether several simultaneous positive urtica tests are all specific, *but must repeat them singly,* and it must be taken into account that the patient, even if his respiratory, or gastrointestinal allergy is not usually accompanied by urticarial eruptions, may have haematogenously circulating antigens from his natural exposure to the allergen, and that this is the cause of an apparently

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From the Central Hospital, Medical Department, Örebro, Sweden
(Chief: H. Malmros, M.D.)

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During the past few decades the chemical industry has presented us with a large number of very valuable drugs. Many of them are highly active, chemically and biologically, and have a capacity for sensitization. These drug factories produce tons of tablets of various kinds, and the public is becoming more and more in the habit of taking them. Therefore it is not surprising that more and more cases of drug allergy are brought to our notice.

This allergy manifests itself in various forms, such as asthma, rhinitis, abdominal pains, headache, fever, urticaria, Quincke's oedema, exanthema, eczema, exfoliative dermatitis, fixed drug eruptions, agranulocytosis and purpura. Sensitization may proceed as a result of both peroral and parenteral administration and also when the drug is employed externally. Various forms of allergy can be produced by the same drug. In some cases, for instance, sulpha preparations may case exanthema with or without fever, and in other cases agranulocytosis. On the other hand, there may be skin changes of exactly the same appearance caused by several different chemical preparations, for example a fixed drug eruption from phenolphthalein, antipyrine, pyramidon or quinine. Diagnostic difficulties are increased still more by the fact that the same or similar symptoms may appear in other diseases which are unconnected with drug allergy. It is often difficult to decide

culin reactivity can be excluded; the investigations of the so-called positive-anergic tuberculids have proved this. The tuberculin test may also be *falsely positive* (so-called parallergic) on account of another infection-allergic reaction taking place simultaneously in the organism.

Just as the strength of an eczematous epidermis allergy cannot be judged by the extent of the reaction to a test with a certain concentration, but is expressed quantitatively by the threshold value of the reaction, much more valuable information (pathogenetically and diagnostically) has been obtained from the *threshold value* of the intracutaneous tuberculin reaction than from the reaction strength to a couple of definite quantities of tuberculin. Again in allergometry on the basis of urtica tests the threshold value alone seems to give useful information; the size of the reaction is much more subordinate.

In eczema tests consideration must be given to *"negative phases"* in the allergy immediately after large eczema eruptions. The tuberculin reactivity can also be affected transitorily by the immunological state of reactivity of the organism. Similar care must undoubtedly also be taken when making examinations with the urtica test.

In presenting these observations it has been my desire to draw attention to the lessons which allergologists, working mainly with urtica tests, will undoubtedly be able to learn from experience gained from investigations into other forms of allergic skin diagnostics.

In conclusion I would merely recall that not all forms of allergy, not even indubitable allergic skin affections such as fixed drug erythema and lichen ruber-like neosalvarsan exanthema, can be demonstrated by known allergic skin tests. *We must not limit the problem of allergy to affections capable of being diagnosed by skin tests, let alone urtica tests.*—We must also remember that it is not only the skin that has limited possibilities for various forms of reactions, and that in this respect the urticarial reaction itself may be due to many pathogenetic mechanisms; indeed, spontaneous urticaria is only relatively rarely of allergic origin. What is more, the organs which are the seat of allergic affections, in whose

diagnosis the urtica test may be of so much importance, have only few different possibilities of reacting; and because a reaction *can* be excited by the allergic mechanism, it need not by any means *always* have that pathogenesis.

DISCUSSION: P. H. NEXMAND, C. SONNE, E. B. SALÉN

Nexmand, P. H.: I thank Dr. Salén for his very interesting paper.

There is something tragic in the circumstance that at this first Scandinavian congress of allergologists we have to place the diagnostic and clinical value of cutaneous tests at the head of the agenda—thirty-five years after *Schloss* introduced this method of diagnosis into the clinic. This suggests that something is wrong and that our diagnostic methods within the allergy discipline are unsatisfactory.

It was already in 1922 that *Rackeman* drew attention to the scientific risk involved in incorrect evaluations of the reactions and, very prophetically, he wrote that a liberal interpretation of cutaneous tests would engender confusion and be an obstacle to scientific allergy research.

In the few minutes allotted to me I should like to bring up the question: Should we use intradermal tests or scratch tests?

It is generally said that intradermal tests are about a hundred times more sensitive that scratch tests. But after making both intradermal and scratch tests with the same dilutions I have formed the opinion that the two methods are quite equivalent, *qualitatively*, irrespective of the dilution, provided sufficiently critical criteria are employed in evaluating the results, or, in other words, that the reactions are judged *not* by means of a finely-graded millimetre scale, but by the urticarial quality of the reactions.

When we employ 0.01 ml. for intradermal tests we obtain reactions which—quantitatively as well—agree with those obtained from scratch tests; the much too frequent use of 0.1 ml. results in a difference in the size of the reaction, measured in millimetres, but the sensitivity with regard to whealing is the same.

I consider it a natural assumption that the "positive" reactions appearing in intradermal tests with allergens which are incapable of giving positive reactions in scratch tests with dry

extracts or highly concentrated solutions, must be regarded as *pseudo-positive* and therefore worthless.

Another question is: which of the two methods is the more suitable, histologically. In scratch tests we produce a superficial

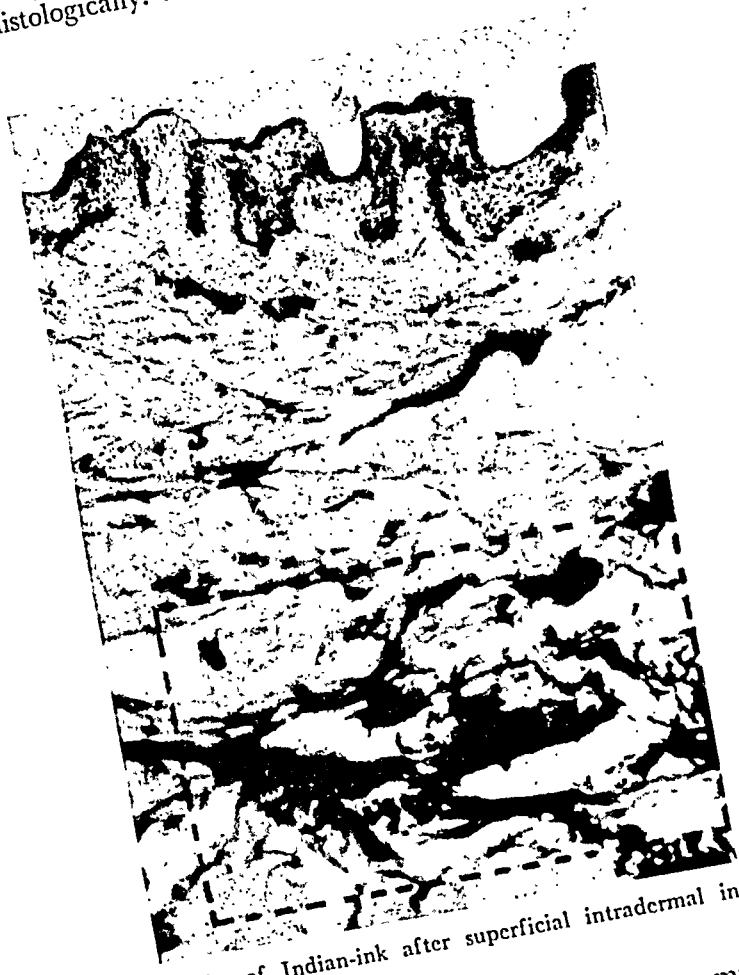

Fig. 1. Site of Indian-ink after superficial intradermal injection.

lesion in the epidermis and derma, and the allergen must of itself diffuse down to the shock organ, i.e. the capillaries in the papillary layer of the dermis and in the sub-papillary network. The reaction may be masked by traumatic reaction, though this will be fairly limited. In intradermal tests we inject a quantity of fluid deep

down in the dermis, much deeper down than is generally thought. From there the allergen has to diffuse upwards through the barrier that is formed by the sub-papillary network before it can come into relation with the papillary capillary loops. The injected fluid exerts a traumatic effect in all directions on capillaries, arterioles and lymph vessels. Depending upon the individual reactivity to traumatic influences, this traumatic effect is capable of inducing a falsely positive reaction and may perhaps conceal a really positive, weak reaction. The greater the quantity of fluid, the greater the trauma.

How deep down the fluid injected intradermally is placed will be seen from the accompanying microphotograph. A thin suspension of Indian ink was injected by the usual method, the needle being visible through the skin after being introduced. It is surprising to see how distantly the injected ink depot lies from the epidermis and the dermis papillae.

Sonne, C.: I should like to point out that an asthmatic respiration is presumably due to a contraction in the bronchioli, and that it is not certain that a contraction of this kind is exclusively the result of allergic influence. For example, there can scarcely be any doubt that a purely psychic influence may occasionally induce it, and purely mechanical changes can do so. When one lies down it is reasonable to think that a greater volume of blood will flow into the lungs; this may cause some swelling of the bronchial mucosa and thereby perhaps secretion. In other words, on the whole a contraction. This is very easy to demonstrate on many bronchitis and asthma patients who may not have rhonchi in the erect posture, but who get it in a few minutes when they lie down; sometimes the difficulty increases to a veritable asthmatic paroxysm when they have been lying for some time. Once we are aware of this fact, it is easy to imagine that in it we have the natural explanation of why attacks of asthma are so frequent at night. Exertion, and undoubtedly anxiety too (psychic influence) cause much fluctuation of the bronchial mucosa and consequently contraction.

Naturally, no one doubts that attacks of asthma may be allergic; but to my mind it is definitely wrong to think and act as if every attack were governed by allergy.

Salén, E. B.: Of the contributions to the discussion I think Professor Sonne's was the most important in principle. As at the Northern Congress of Internal Medicine in Helsingfors about ten years ago, Professor Sonne today again directed his criticism against the exaggerations which modern allergy teachings may nourish in their bosom. Professor Sonne seems inclined to accord psychogenic causal factors a greater role than allergy enthusiasts do. It need hardly be pointed out how in particular paroxysmal asthma must be calculated to produce nervous perturbation in a patient. Unawares, fitfully, he is struck at any time by perhaps a severe attack like a bolt from the blue. From being in the best of health he is suddenly brought to a state of severe illness. Such a patient often has stubborn difficulty at night which deprives him of sleep. All this will certainly help to *bring on* nervous emotion, even in the case of a person whose nerves are otherwise quite in order.

One who is occupied with these diseases has opportunities almost daily of observing how even slight allergic manifestations *secondarily* are capable of involving nervous symptoms. To give an example: a lad attending school in the exacting third form had always been exemplary and never neglected his studies; he began to be "slipshod", seemed "nonchalant", his school work was poor; he gave the teachers and his parents the impression of being nervous, irritable, not like he was before. The only morbid or abnormal symptom noticed was a fairly heavy, troublesome rhinitis of the *morning* type. It was said to have set in with a cold some months before. When examined he presented a normal sedimentation rate, moderate blood eosinophilia. The antigen analysis resulted in fairly strong reactions with dust and bacterial extract. Nasological examination revealed an ethmoiditis, which was treated operatively. Partial dust desensitization was carried out and the patient was given an allergol cure. After two or three weeks he was free of his symptoms, had no trouble. The psychic change, the nervous symptoms, had disappeared. To his teachers and relatives he is now the same ambitious youth as before.

At the Helsingfors congress I cited as an instructive example the case of hay-fever patients. If one has the chance of observing a number of these *out* of the season, the impression gained is that usually they are individuals without psychic blemish or

particularly nervous tendency. Then, if they come into the hay-fever season and are much affected, one finds in the *majority* of them marked nervous disorders, indeed sometimes such a distinct psychic inversion that it leads to attempted suicide. When the season is over, we find the same normal individuals as before: self-respecting, almost 100 per cent. able-bodied people.

On turning to the selected, highly dust-hypersensitive group of asthmatic children cared for at the Skolhemmet in Stockholm, we find the following: As a rule the most severe cases, or at any rate the severe ones, are taken from the elementary schools of Stockholm. This also means that these children have previously been treated by a number of doctors who, all according to their several attitudes, prescribed asthma medicines, opium cough medicines, antinervina, etc., all without much palpable result. Many of these children, probably the majority, have been treated for one or more periods at various hospitals, usually pediatric, likewise without much benefit. The psychic trauma to which these children are exposed by being taken from the family environment and transplanted to the Skolhemmet must be obvious. Notwithstanding this we find (see Salén, Hulting & Nordenfors) that there—in dust-cleared surroundings and under rational anti-allergic treatment—they quickly become free of symptoms and trouble, behave like healthy children, go in for sports. In fact, the majority of them were even able to do the so-called community-walk in good time! From having to be absent from school perhaps half or more of the term, absence due to asthma hardly occurs at all!

From the work I have mentioned as well as from several since then on children's asthma (see Salén, Flensborg, E. Bruun, etc.) it will be seen that by these means we have been able to progress to a highly marked change in the prognosis in a favourable direction.

As a reason for his attitude Professor Sonne said that inhalation tests with dust in similar cases had been negative. This calls for the indication of some facts. Bed material from the dwellings of dust-hypersensitive asthma patients usually gives a biologically highly active extract. Even so, night attacks most often occur well into the night, or sometimes in the early morning. In the many tests that were made of letting a child from the Skolhemmet, after being free from attacks for some time, go home for a few days

it was found that there often was no trouble the *first* night; the attacks returned only on the second or third night. This and other observations thus seem to argue that it often requires several hours' inhalation before the effect comes. This must certainly be given full consideration when judging Professor Sonne's negative exposure tests.

When carrying out specific desensitization it is nothing unusual for allergic symptoms to be produced; this despite the fact that one begins with quite a small quantity of a dilution that is undoubtedly tolerated by the patient, whereafter the dose increases very slowly. There can hardly be any question of these provoked symptoms being psychogenous. If that were the case, they would doubtless *appear in conjunction with the first injections,* before the patient had become accustomed to the injection treatment. In actual fact, however, this is hardly ever the case. As a rule they make their first appearance towards the end of the treatment, i.e. with the large doses, the more concentrated solutions.

Furthermore, according to the psychogenicity hypothesis the necessary presupposition would be that the clinical manifestations thereby produced in an asthmatic person would take the form of an *asthmatic* reaction. *In many cases, however, this is not so.* An asthmatic while being desensitized specifically will not uncommonly present quite another allergic symptom complex during the treatment, a complex *which he has not previously experienced,* for instance urticaria, Quincke's oedema or the like. Thus it is obvious that that there can be no question of psychogenic excitation.

This is verified so much the more by the circumstance that in these reactions we sometimes see clearly objective changes, e.g. in the form of a marked rise in the sedimentation rate, in one case up to 80 mm./hour in the course of a few hours, followed by a drop within a very short time.

To some extent the cause of Professor Sonne's attitude to these matters may be partly that the asthma material in an ordinary medical department usually consists chiefly of old, inveterate cases, generally with secondary complications, relatively often with poor or negative skin spectra; that is to say, cases that are difficult or impossible to make out from an allergy point of view. As Arner and I shall endeavour to show at the Congress, these

cases are almost always *complicated with infection*, in many instances probably an expression of a bacterial allergy in which rational infection clearance, in conjunction with the necessary anti-allergic treatment, may work miracles. This brings me to another question: How will the psychogenic interpretation explain that some of these infection-complicated cases are allergic to aspirin, not infrequently to such a degree that a few milligrammes of the drug may lead to death?

Our view of these questions is that the nervous disorders sometimes occurring in asthma etc. are usually *secondary*. The question with which I should like to replace Professor Sonne's is: In the case of old, chronic asthmatics, can *unspecific* (that is to say non-allergic) influences cause an exacerbation of the asthma, excite an asthma attack? Vaughan, who takes a special interest in these matters, considers it possible and imagines the functional mechanism as a conditional reflex ad modum Pavlow, with psychogenic factors as a background. Vaughan's idea seems acceptable and attractive. Our own opinion is that we should give particular consideration to the conception of *exacerbation*. The fact is that in such cases we find very often that patients more or less permanently go about with what we have previously categorized as a *subasthma:* the subjective symptoms in quiescence are insignificant or absent, in auscultation one can hear ronchi only with forced, deep exspiration. Dyspnoea occurs only with exertion. In these very cases we also find the condition indicated by Professor Sonne, that the patient has increased difficulty and increased objective symptoms of ronchi etc. in the *recumbent* posture. In such cases the patient is sensitive to almost everything: fog, wind, cold air, road dust, smoky air, etc., all of them factors which the asthma patient tolerates almost just as well as a healthy individual *once he gets rid of his sub-asthma*. It is surely fairly obvious that in such cases psychic irritation will also cause an *exacerbation*.

The second fundamental question touched upon in the discussion is whether cutaneous or intracutaneous tests should be made. It is considered that intracutaneous tests are more circumstantial, more expensive and require more apparatus, etc. I would say that both methods of testing are equally efficacious, *provided that all*

necessary precautions are taken. In my opinion—in which I disagree with a number of speakers—the intracutaneous test is quicker, it is easier to perform uniformly, and therefore more certain in the matter of judging the result and avoiding wrong conclusions, than the cutaneous test. For an allergy clinic or a specialist, having to test several cases at the same time, it is beyond doubt the more practical. And I am quite convinced that it is preferable for titration. Turning to the general opinion as expressed in recent American literature, we find that the majority of authors agree with this point of view, whereas the cutaneous test has a certain preference among colleagues who rarely occupy themselves with antigen analysis.

The circumstance that the cutaneous test is 100 to 1000 times less sensitive than the intracutaneous, seems to me should be placed entirely to the credit of the latter. The opposite opinion can be defended *only if it is considered obvious that wrongly standardized extract will be employed* or *that necessary precautions are ignored.* The control of the serological specificity of a reaction is also easy. For the rest I take the liberty of referring to what was said previously regarding the evaluation of the weak skin reactions.

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From the Central Hospital, Medical Department, Örebro, Sweden
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This allergy manifests itself in various forms, such as asthma, rhinitis, abdominal pains, headache, fever, urticaria, Quincke's oedema, exanthema, eczema, exfoliative dermatitis, fixed drug eruptions, agranulocytosis and purpura. Sensitization may proceed as a result of both peroral and parenteral administration and also when the drug is employed externally. Various forms of allergy can be produced by the same drug. In some cases, for instance, sulpha preparations may case exanthema with or without fever, and in other cases agranulocytosis. On the other hand, there may be skin changes of exactly the same appearance caused by several different chemical preparations, for example a fixed drug eruption from phenolphthalein, antipyrine, pyramidon or quinine. Diagnostic difficulties are increased still more by the fact that the same or similar symptoms may appear in other diseases which are unconnected with drug allergy. It is often difficult to decide

whether a small-spotted rash is caused by scarlatina or it is a drug exanthema, whether an asthma is caused by dust allergy or aspirin, an erythema nodosum eruption by sulphathiazol or tuberculosis.

If a patient be asked if he has used medicine, he often replies in the negative. A headache powder or a laxative pill is rarely considered as medicine by a patient. Patent medicines with fancy names may often conceal dangerous chemical substances; I need merely mention the once popular tablets with names ending in "-on", which proved to contain amidopyrine and therefore often induced agranulocytosis.

The diagnosis often being very difficult to make, skin tests have been resorted to in order to get on the track of the offending drug. In actual fact it was in a case of mercury dermatitis that *Jadassohn* in 1895 first employed the patch test diagnostically. In 1917 *Boerner* described two cases with a positive skin test in quinine hypersensitivity.

Since the patch test and other skin tests began to be used more generally in diagnostics in allergic diseases, a large number of drug allergies have also been examined by these means. In the literature we find very varying reports on the results. In sulphonamide hypersensitivity, for example, positive skin tests are described by *Goodman & Levy, Myers* et al., *Salvin,* and others. Most authors, however, consider that the skin test gives unsatisfactory results, in that mostly they are negative, even when the case is definitely one of drug allergy. There are numerous descriptions of cases where, to intracutaneous tests with dilute and also relatively concentrated aqueous solutions of various sulpha preparations, the results have been completely negative, although a strong allergic reaction could be produced by giving the patient a fraction of a sulpha tablet per os.

The cause of this lack of conformity between skin test and clinical course is not quite clear. The complications which may appear in conjunction with sulpha treatment are not all of equal value from the point of view of allergy. It is credible, for instance, that acute haemolytic anaemia is the result of a direct toxic influence by sulpha and that is entirely unconnected with allergy.

This may explain why *Harvey & Janeway* found negative skin tests in such cases. *Park* points out that there are two types of sulphonamide allergy. One form occurs whether the drug is administered by mouth or as an injection. The following symptoms may then be observed: fever, skin eruption (often of morbilliform type), leucocytosis, joint pains, or sometimes agranulocytosis. The other form appears when the preparation is applied externally and has the form of a dermatitis. Such cases are so very highly sensitized that a recrudescence of the process occurs if a minimal quantity is ingested. With this type of sulpha hypersensitivity a positive result may be expected from a patch test (e.g. with a 50 % paste). In cases where sulpha is administered by the mouth and produces an exanthema and possibly fever, the skin test is considered to give a most uncertain result, no matter whether the method be the patch test, the scratch test, or intracutaneous test with aqueous solutions of various concentrations. The cause of this reaction failure has been thought to be that sulphonamide and other chemical preparations employed as drugs do not as such have a directly sensitizing effect, but only after having formed in the organism a chemical combination with other substances, for example the body's own protein. According to the hapten theory based upon *Landsteiner*'s fundamental researches, a large number of chemical substances can be transformed into fully valid antigens by combination with certain proteins. Experimental research has verified that this really is so. *Haxthausen,* by injecting horse, sheep, goat or guinea-pig serum together with mercury salts, chromium salts or formalin, succeeded in producing in man a cutaneous allergy to these simple chemical substances.

The obvious course was to try to utilize this knowledge gained experimentally for clinical diagnostic purposes. A year or two ago, when we had some cases of sulpha hypersensitivity hospitalized in our department of the Central Hospital at Örebro, the question of the usefulness of the skin test presented itself. Intracutaneous tests made in the usual manner with sulpha preparations in aqueous solution gave uncertain results. In some cases we obtained distinctly positive reactions, even when highly diluted solutions were employed, but generally speaking the pa-

tients did not react. It then occurred to me that it might be possible to secure positive skin tests if the sulpha preparation were mixed with a protein solution, for example serum. It is true that in his experiments *Haxthausen* had not succeeded in obtaining sensitization when using human serum, but only when he added the active substance to serum from animals. To my mind, however, it seemed inadmissible to use foreign serum for tests on patients. We therefore employed the patient's own serum with the addition of different sulpha preparations in varying quantities. Before use this sulpha-serum compound was allowed to stand for 24 hours in the refrigerator. The results of these experiments varied. In two cases we got positive intracutaneous reactions with sulpha serum in highly diluted form, although in aqueous solution sulpha gave a negative result. In other cases, however, all the skin tests were negative. Thus the method can be used, but from a negative test one cannot conclude that the patient is not sensitive to sulpha.

By a closer search of the literature I have since found that the same or similar methods were employed by other workers. *Dameshek* & *Colmes* already in 1936 described three cases of amidopyrine agranulocytosis which gave positive intracutaneous reactions to amidopyrine mixed with the patients' own serum. On the other hand, amidopyrine in aqueous solution gave a negative result, as also the patch test. *Schlesinger* & *Mitchell, Wedum,* and *Abernethy, Bukantz* & *Minor* have employed azoprotein combinations of sulpha preparations made from human serum by *Landsteiner*'s method, but without succeeding in obtaining positive skin reactions.

I myself recently had a case of amidopyrine agranulocytosis which showed negative intracutaneous reactions both with amidopyrine in aqueous solution and with amidopyrine dissolved in the patient's own serum.

In 1944 *Leftwick* described another method for intracutaneous tests in sulpha hypersensitivity which, in his opinion, was very reliable. On testing 30 cases of sulpha hypersensitivity he had positive intracutaneous tests in 28. For these tests he employed serum from another patient who had been given the full

dosage of the sulpha preparation for at least a week. It is true that the sera employed were checked for W. R. and bacterial sterility, but nevertheless the method is not a very attractive one, because one cannot rule out the possibility that hepatitis and other virus diseases may be transmitted through the intracutaneous test. (The same element of risk is connected with *Prausnitz-Küstner*'s test.) Moreover, in my control tests it has been found that normal serum kept for a couple of weeks in the refrigerator often gives a positive control. On the whole, it is very difficult to judge intracutaneous tests with serum, since absorption is very slow and the bleb often remains half an hour or more.

It is accordingly evident that none of the methods hitherto employed for skin testing in drug allergy are fully reliable. On the other hand, for those forms of drug allergy that are manifested under the guise of a dermatitis it is reasonable to anticipate a positive result with the ordinary patch test. This holds good above all in cases where sensitization has come about by the use of the drug externally, e.g. for the local treatment of an infected eczema, a wound or a burn. Even when the drug is administered by the mouth or parenterally, an eczematous process may form in certain instances. The patch test will generally also be positive in the case of dermatitis similarly induced internally. In other cases the skin test is untrustworthy, as already stated, no matter whether one employs the patch test, the scratch test or the intracutaneous test with aqueous solutions or serum.

Furthermore, the use of diagnostic skin tests and exposure tests is not always free of danger. In this connection I would utter a warning against the use of the intracutaneous test on asthma patients suspected of aspirin hypersensitivity, as it may involve fatal shock. Nor should the exposure test or patch test be employed in exfoliative dermatitis; indeed, even with other forms of dermatitis it is advisable to observe caution and, for example, make no test as long as the patient still has widespread skin changes. As always in medicine, the patient must not be subjected to unnecessary risk.

In actual fact, in the majority of cases a quite reliable diagnosis can be arrived at if only one goes carefully into the

history and follows the clinical course. The medical practitioner should first and foremost always have drug allergy in mind whenever he sees a curious skin eruption or any other unusual disease picture and the patient does not respond satisfactorily to treatment. Once the idea of drug allergy is established, the diagnosis is not so difficult as a rule and the therapy follows as a matter of course. Instead of saying to the patient "take this medicine and you will be well," one need simply say "stop taking those tablets and you will get better."

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A CASE OF SALVARSAN ASTHMA ACCOMPANIED BY JOINT TROUBLES DUE TO LERGITIN

By

C. E. SONCK,
Helsingfors.

Salvarsan asthma is a phenomenon rarely encountered. In his book *"The modern treatment of syphilis"* (1944)), *J. E. Moore* says: "We have never seen such an incident, but have seen asthma and urticaria occuring in a pharmacist from the inhalation of tryparsamide." In the great American arsphenamine statistical works—e.g. *Cole* & collab. (75,000 patients, 8,810 of which showed various subsidiary effects) or *Phelps* & *Washburn* (in all 272,354 injections)—no cases of asthma are mentioned. *De Beyrouth* (1920) and *Mibelli* (1927) have described one case of asthma each, caused by intravenous injections. *Klauder* (1922), *Vuletic* (1933), *Szarvas* (1934), *Cerri* (1936), *Saunders* (1942), *Quero Morente* & *Gómez Orbaneja* (1942) and *J. W. Thomas* (1943) enriched medical literature with single cases, in which asthma and sneezing occurred in doctors and pharmacists, partly through inhalation of salvarsan (arsphenamine) in powder form, partly through the skin of the fingers coming into contact with salvarsan solution.

Klauder's case concerned a doctor who had earlier handled arsphenamine without detriment. The allergy became manifest 8 months after his having volunteered as a control subject in a study of cutaneous tests with arsphenamine. On this occasion a subcutaneous injection of diluted arsphenamine solution caused an indurated area that remained for several months. *Klauder* especially accentuates the importance of the sensitizing effect

of arsphenamine in the tissues (e.g. by paravenous injection). In this case of asthma positive scarification tests with a 2 per cent solution of arsphenamine and a 2 per cent solution of neo-arsphenamine flared up again later on, when the patient tried to desensitize with small doses of arsphenamine of 0.0001, 0.001 and 0.01 g. perorally.

Thomas has reported the case of a doctor who had earlier had urticaria and angioneurotic oedema and was hypersensitive to several different kinds of food, and who suffered from atopic and contact dermatitis, vasomotorial rhinitis, migraine, gastro-intestinal allergy and bronchial asthma. He experienced symptoms of asthma about 2 minutes after having opened an ampule of neoarsphenamine. In order to find out a possibility of oral desensitization he took some cc. of neoarsphenamine solution (about 3 mg.) orally. Twenty minutes later he was in an asthmatic condition with cyanosis of such severe and obstinate character that his life could be saved only with great difficulty.

I am now going to relate a typical case of salvarsan asthma in a female doctor, who also suffers from allergic salvarsan eruptions. I want to point out at once that in this case the antihistamine preparation Lergitin, but not salvarsan, provokes quite marked joint troubles with stiffness and swelling.

This colleague (born 1896) has a very marked hereditary tendency to hay fever, asthma, infantile eczema and Besnier's prurigo in the family. Her sister and daughter as well as all cousins on her father's side are allergic. As a child she suffered herself from infantile eczema and in her youth from Prurigo Besnier. After 1923 she was, however, free from eruptions for about 15 years. As a young schoolgirl she had for some years also, nightly attacks of asthma.

In the year 1929 (having been for a period of 20 years quite free from asthma) she became a doctor in the Dermatological Hospital, where she came into daily contact with salvarsan. She now began to suffer from difficult attacks of asthma and almost every day had to take an adrenaline injection in the clinic. It was finally found out that these attacks of asthma were provoked by the salvarsan. She often experienced the sensation of asthma almost immediately after the opening of an ampule of salvarsan. The German preparation Neosalvarsan proved to have the strongest effect, the corresponding English preparation and the French Novarsenobillon especially seem to have been somewhat milder. Recently she has observed that she gets asthma

also from the new English oxophenarsine preparation Neo-Halarsine (arsphenoxide-tartrate), and not only therefore from salvarsan (arsphenamine) compositions proper. She has noticed no hypersensitivity to ether-vapours.

The hypersensitivity to salvarsan is especially manifest when the patient comes into contact with salvarsan through rhagades in the skin. There develops a violent anaphylactic reaction some minutes after the salvarsan has entered the wound. It begins with a feeling of cramp in the region of the diaphragm and is almost immediately followed by a strong congestion of the face and severe asthma. In these attacks caused through wounds in the skin—which are the worst to the patient—the asthma is localized, as it were, deeper in the chest than in the attacks provoked by inhalation. In the initial stage the sensations of dyspnoea and anguish are very strong. The patient feels that she is going to die. The cramp is followed by an abundant secretion of slime with coughing and rattle. Big, red, urticarial wheals, accompanied by intensive itching, develop in the whole face and around the anus. Disseminated efflorescences also occur on the buttocks and on other parts of the trunk, while the extremities are affected to a much smaller extent or not at all. With the help of an adrenaline-injection this reaction subsides within about 2 hours. (As the difficult symptoms have always forced the patient to take to adrenaline, spontaneous subsidence could never be observed.)

On one occasion it happened that a small quantity of the prepared salvarsan solution splashed into her eye. It caused a violent allergic conjunctivitis with intense redness, itching and flow of tears as well as a strong swelling of the eyelids, but no asthma. The ocular reaction remained for a couple of days.

The patient has been able to protect herself against the effects of salvarsan caused through rhagades in the skin by using rubber gloves. It has been more troublesome completely to avoid inhalation of salvarsan vapours. The patient has been obliged to open the ampules under water or in a fume cupboard. Asthma-attacks caused by inhalation of salvarsan in powder form begin with a feeling of cramp in the trachea—thus somewhat higher up—and are generally easier, or in any case less disagreeable than the other attacks described above. Also in these cases the cramp is followed by a phase of secretion of slime and rattle.

The patient has, queerly enough, noticed no sneezing or vasomotor rhinitis in connection with salvarsan. But she reacts in this way to cats. (She has been hypersensitive to cats since childhood.) It may also be pointed out that she has noticed no allergy whatever to food.

In the year of war 1941, when the patient more than ever had to work with salvarsan, and besides felt tired and exhausted, she had an eczema, evidently also caused by salvarsan. It began in May 1941 in the face, then spread to the hands, the arms and the throat and finally also, to a certain extent, to other parts of her body. It healed completely in the middle of July, when she had her holiday. When she recommenced work in the autumn

the eczema flared up again, and in this way it has now been going on for more than 6 years.

This salvarsan eczema is partly erythematous, partly papulo-urticarial or —especially on the hands—eczematously oozing and strongly itching. It is worst in the beginning of the autumn, i.e. after the patient's renewed contact with salvarsan after a holiday. Later on a somewhat more quiet stage sets in, as if a certain desensitization had taken place. Towards spring—after the exertions of the winter—the eczema again becomes worse. When the patient goes to her country-place in the summer it doesn't take more than a couple of days before she suddenly feels better, quite a different person. The eczema disappears completely in the course of the summer.

In September 1946, the salvarsan eczema being especially painful, the patient was for some days admitted into the Dermatological Clinic. On this occasion chamber-tests with ordinary neosalvarsan-solution (dilution of 0.45/10 cc.) and with rubber were carried out. These tests were applied on a spot of the thorax where the patient had never had an eczema. When removed 24 hours later a clearly positive reaction to neosalvarsan was noted, while the skin on the place of the rubberpatch only showed an indistinct, almost negative reaction. The test reaction to salvarsan subsided within a few days.

On later exacerbations of the eczema there was a flare-up of the salvarsan-test simultaneously with the eczema itself. This occurred on repeated occasions in the course of at least half a year. The reaction on the place of the test, however, always remained strictly circumscribed. On the place of the rubber test no reaction was observed.

The patient has never taken salvarsan perorally. Judging from certain observations described in the literature (*Thomas'* case), such an experiment is not recommendable.

In the blood pictures, few in number it is true, that have been examined, there has never been evidence of eosinophilia, not even during the worst periods of asthma. The blood count on the 1st of Oct., 1947 showed: Hemoglobin 74/85 per cent, erythrocytes 4,320,000, color index 1.00, leukocytes 6,000, with segmented polymorphonuclear leukocytes 72.5 per cent, non-segmented 1.5 per cent, lymphocytes 16.0 per cent, monocytes 10.0 per cent and eosinophils 0 per cent. Sedimentation rate 10/24 mm.

Therapeutically allergol and heparin were of no effect, calcium had a certain, but small effect. Adrenalin was of the greatest assistance. No systematic desensitization has been tried.

Hoping to get some relief for her salvarsan allergy by antihistamine-effect, the patient took by mouth Lergitin-*Recip* (N'-dimethyl-N''-benzyl-N''-phenyl-aethylendiamin. hydrochlor. 0.10) tablets, which were reputed to be effective in cases of drug eruptions. She began the treatment in the spring of 1947 taking one tablet 4 times a day, without noticing any subsidiary effects at first. In September 1947, the salvarsan asthma having recurred, the patient again took to Lergitin tablets, one tablet 4 times a day. After about 10 days,

however, she experienced a growing sensation of stiffness in the joints as well as increasing rheumatic pains and tenderness, especially in the wrists and shoulders, but to a certain extent also in the hips, knees and ankles. The stiffness was worst in the mornings. The affection of the joints seemed to show rather great similarities to e.g. a bismuth-rheumatism. Both hand-joints were visibly swollen. The patient was obliged to interrupt the Lergitin treatment. At this the joint-troubles disappeared within some two or three days. Some days later, feeling nothing whatever in her joints, she took one evening, as an experiment, one single tablet of Lergitin, with the consequence that she woke up the following morning with stiff and rheumatic joints. The hand-joints also on this occasion displayed a swelling of a temporary nature. After this she successfully continued with Deseryl-*Leo*, taking one tablet 4 times a day peroraly, later also with Benadryl and Amidryl-*Medicinalco* (β-dimethylaminoethyl benzhydryl ether hydrochloride 0.05 g.). They all had an alleviating effect on her salvarsan allergy, especially on the eruption, without causing joint-troubles. Antistin-*Ciba* (2-phenylbenzyl amino methyl-imidazolin. hydrochlor. 0.10 g.) could also be used without causing joint-troubles, but proved to be less effective. It is of special interest that Pyribenzamine (N,N-dimethyl-N'-benzyl-N'(α-pyridyl)-ethylenediamine monohydrochloride), the formula of which is like that of Lergitin, the α-pyridyl group excepted, caused no point troubles. It proved, however, to be without benefit to the eruption.

One evening in the beginning of February 1948, when she happened to have no other tablets, she again made an attempt with two tablets of Lergitin. She did not have to wait long for the results: The following morning her joints were stiff and swollen again. Thus it seems obvious that the joints of this patient are allergic to Lergitin.

SUMMARY

Report of a case of salvarsan asthma in a female doctor. The asthma is provoked not only by inhalation of the salvarsan powder, but also through the skin, especially if some rhagade in the skin happens to come into contact with the salvarsan solution. In the latter case there is a violent anaphylactic reaction, beginning with a feeling of cramp in the region of the diaphragm, followed by strong congestion in the face and severe asthma. On inhalation the attack begins with a feeling of cramp higher up in the trachea. The asthma is accompanied by urticarial eruptions and itching but not by joint-troubles. A small quantity of salvarsan solution that once splashed into her eye caused a violent conjunctivitis without asthma. Especially on the hands there has gradually de-

veloped a salvarsan eczema, which disappears when the patient ceases to handle salvarsan. The skin showed on testing with salvarsan positive test reactions, which, besides, displayed spontaneous flare-ups on later exacerbations. The antihistamine-preparations Deseryl, Benadryl and Amidryl have proved to be of some value in this case, Antistin was less effective. These preparations, as well as Pyribenzamine, could be used without subsidiary effects. Lergitin, (N"-dimethyl-N"-benzyl-N"-phenylaethylendiamin. hydrochlor. 0.10), however, promptly provoked severe allergic joint-troubles.

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ASTHMA

NOMENCLATURE — DEFINITION — PRE-ASTHMATIC STAGE

By

K. H. BAAGØE,
Kolding, Danmark

Nomenclature.

The term asthma has had different meanings in the course of time. Originally "asthma" was used simply in the sense of difficulty in breathing and was used of practically any disease that was accompanied by some form of dyspnoea. Thus it was common to speak of asthma toxicum, diabeticum, carcinomatosum, pneumonicum etc.; *Siegel* (9) states that he has found over twenty similar names in the literature. Nearly all these have passed out of our nomenclature, but a reminiscence of that time is found in the terms "asthma cardiale" and "asthma bronchiale".

The question is whether the time has not come to give up the word asthma in the sense of dyspnoea and abandon the term "asthma cardiale," and exclusively use "asthma" for the so-called "asthma bronchiale."

Definition.

How are we to define asthma? Most definitions hitherto used lay the main emphasis on the paroxysms of asthma. So did I, when in 1926 I defined asthma as a disease characterized by attacks of dyspnoea attended by wheezing and occurring at free intervals—a definition that closely resembled one of the earliest, that made by Salter (7) (1868).

But the question is whether with our present knowledge of asthma it would not be possible to find a more adequate definition.

As recently emphasized by *Kobro* (6) the fact is that in asthmatic bronchitis there are frequently no free intervals between the attacks, indeed in some cases one can hardly speak of attacks in the proper sense of the word, but rather of exacerbations of a more or less permanent state of breathlessness.

So we have every reason to look round for another definition.

Werner Schultz (8) (1924) defined asthma as a state of breathlessness occurring paroxysmally or more continuously, and developing with signs of obstruction of the smaller bronchial tubes.

In recent years similar definitions have been proposed. Thus *Egon Bruun* (3) (1945) defined asthma as a functional multiple bronchiolostenosis, and *Cooke* in his latest book (1947) defines it as a disease due to general bronchial obstruction.

Bruun's definition of asthma as a functional, multiple bronchiolostenosis seems to me to fit in very well with our present knowledge.

The question is whether this definition might be further specified by an addition referring to the etiology or pathogenesis of asthma.

As to etiology, some allergists hold that asthma is always due to allergy, and that accordingly allergy ought to enter into the definition. Others—and perhaps the majority—certainly allow allergy an important place, but do not consider it proved that asthma is always due to allergy.

Another view formerly prevalent was that asthma is a neurosis, but it has probably few supporters nowadays. To be sure, the neurotic element does play a considerable part in the course of the disease, but asthma hardly ever arises on a purely psychical basis.

Finally there are some writers who, like *Heckscher* (5) in Denmark, attach the greatest importance to defects in the patient's carriage and inefficient respiration, and accordingly treat the asthmatics with special gymnastic exercises, by which they believe new attacks can be prevented.

Thus, as you see, opinions on etiology differ.

As regards pathogenesis, there is general agreement that the

asthma paroxysm is due to an obstruction of the bronchioli—but that is the end of agreement.

Cooke (4) maintains the view, which in a former work I (1, 2) have also advocated, that the obstruction is caused by oedema of the wall of the bronchioli in connexion with the tenacious mucus, which more or less fills up the lumina.

Others, however, hold that the attack is due to a spasm of the bronchioli, a hypothesis which, as *Cooke* writes, originated in the days when asthma was considered a neurosis or a simple nerve reflex, and it has never been proved.

As you see, there is at present very little agreement as to etiology and pathogenesis, so the time has hardly come to make them part of the definition.

As a basis for discussion I therefore propose to define asthma as a disease manifesting itself in paroxysmal or more continuous wheezing respiration owing to a functional, multiple bronchiolostenosis and stethoscopally audible in sibilant ronchi.

Pre-asthmatic stage.

In the following I shall deal with the incipient stage of asthma and discuss at what point of the patient's illness one may, according to the proposed definition, say that his asthma has begun.

Asthma may, on the whole, begin in two ways. Either it begins suddenly with typical attacks in a hitherto apparently healthy individual, or it develops after the patient has had slighter or more severe affections of some kind for varying lengths of time. The latter are the cases I shall briefly discuss.

1. In some patients asthma sets in during, or perhaps more frequently, immediately after an acute infectious disease, such as influenza, pneumonia and the like.

2. In other cases the patient has often for years before the typical asthma attacks set in had a tendency to wheezing respiration during exertion, for instance when cycling up a steep hill or when running fast—but it is only during exertion that the wheezing occurs, and there are no spontaneous attacks at this stage.

3. Then there are many patients who state that for several years before their asthma developed they had a tendency to colds in the head, cough, or regular bronchitis.

a. A number of these patients can be sorted out as a separate group, in which from the patients' description of the disorder it can clearly be recognized as a vasomotor rhinitis, sometimes complicated by sinusitis.

b. Another, smaller, group can be segregated, in which the patients have had severe attacks of coughing unaccompanied by wheezing respiration. These attacks consist of a dry, irritating cough with very little expectoration—the patients often call it a "tickling cough", and the attacks are so violent that they may resemble whooping-cough and be mistaken for it.

c. Finally there is the large group of patients who state that they have had bronchitis every winter for years, in young children often accompanied by fever, or they have had common colds with nasal catarrh and coughing, and only gradually have these catarrhal cases assumed the character of asthma.

In many of these cases it may be difficult to decide at what time the patient's asthma really began. If we keep to the definition that asthma is a universal bronchiolostenosis and do not insist that there should be typical paroxysms of asthma, we must count the beginning of asthma from the time when the patient first had wheezing respiration. Thus we shall say that the patient who has had wheezing respiration on exertion only, had asthma even at that stage.

Similarly in the cases of bronchitis the occurrence of wheezing respiration must be decisive, even if there have been no paroxysms.

The question that now arises is: what is the nature of these cases of bronchitis and catarrhalia before they have assumed an asthmatic character? Are they simple catarrhal infections or are they particular forms, perhaps of an allergic nature?

There is a well known fact which I would like to recall: these recurrent catarrhs seem much more frequent in the anamnesis of asthma patients than among normal persons. Thus we see again and again in a family that an asthmatic child has recurrent colds

for years, while his brothers and sisters have had no more colds than normal.

It seems to me that one must assume either that individuals who later develop asthma have a congenital disposition for common colds which gradually causes the development of asthma, or, what seems to me a more reasonable explanation, that these apparently common colds are allergically conditioned and arise on the basis of a congenital allergic disposition.

I shall give a brief account of the cases of two patients that can illustrate it. Neither of these patients has so far had typical attacks of asthma, but both of them afford examples of just the disorder we see in the pre-asthmatic stage. The first patient has attacks of coughing (ad 3 b).

The patient is a 6 year old boy of an asthmatic family. From the first year of his life he has periodically suffered from severe attacks of coughing, especially in the morning, and according to the statement of his parents they have never been accompanied by wheezing respiration.

The physical examination shows normal lung-stethoscopy, he has considerable cosinophilia in the blood and expectoration, and positive cutaneous reaction to egg (he gets lip-oedema from egg).

As mentioned his lung-stethoscopy was normal, but auscultation has not been made during his attacks of coughing. It is possible that there may be ronchi during his attacks of coughing. I mention this possibility, because I have treated children with a similar anamnesis, in whom auscultation showed ronchi without the patient having audible wheezing respiration and without the parents ever having noticed anything of the kind.

This patient is allergic; he has not yet developed asthma, but his attacks of coughing are just like those so often found in the anamnesis of asthmatic patients. I do not know what is the cause of this cough, but presumably it is due to irritation of the wall of trachea or bronchi, an "itch", perhaps caused by oedema.

This pre-asthmatic, probably allergic, tracheitis or bronchitis must presumably be considered on a level with pre-asthmatic vasomotor rhinitis.

The case of the second patient falls into the category of catarrhs and bronchitis (ad 3 c).

The patient is a boy of 18, his mother suffers from vasomotor rhinitis and has had slight attacks of asthma, and in the words of his mother his anamnesis would read as follows: For ten years periodical, feverish attacks with purulent nasal catarrh and bronchitis, and two slight attacks of pneumonia.

I have watched him closely from birth, so I know him. He himself has vasomotor rhinitis, which however is partly disguised by complicating rhinopharyngitis and sinusitis.

Apart from the two slight bouts of pneumonia, his lung-stethoscopy during his feverish catarrhal attacks has usually been normal. But on a very few occasions—at intervals of years—there were scattered ronchi to be heard and he had a slightly wheezing respiration for one or two days.—The blood contains 10 % eosinophile cells and he has positive cutaneous reaction to feathers and pollen, but has never had symptoms of hay fever.

This boy too is allergic, but his clinical picture is dominated by feverish, catarrhal attacks, and only because I have had opportunities to examine him daily during his periods of illness, has it been disclosed that he suffers from very slight asthmatic cases of a transient nature. But I should think that his infections arise because of his allergic constitution—vasomotor rhinitis and sinusitis.

Thus in my opinion the former was a case of pre-asthmatic tracheitis (or bronchitis) of an allergic nature, the latter a case of pre-asthmatic catarrhal infections, based on his allergic condition.

It is not my intention to generalize from these cases, but I want to call attention to the pre-asthmatic stages, and to raise the question of their nature. As far as I know, no material has been published concerning patients who have been examined in the pre-asthmatic stage. Ordinarily we have only the patient's statement to build on, and that is not sufficient to decide the nature of these disorders.

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From the Medical Out-Patient Department of the Rigshospital, Allergy Consultation, Copenhagen, Director: Professor Eggert Moller, M.D.

A FOLLOW-UP EXAMINATION CONCERNING SPECIFIC DESENSITIZATION OF ASTHMA PATIENTS

(*Preliminary report*)

By

ERIK HENRIKSEN

Specific desensitization of asthmatic patients has been used at some clinics for several years. The value of the treatment is, however, still discussed. It must therefore be considered of importance if reliable criteria of the efficiency of the treatment can be obtained.

Since 1941 asthmatic patients have been treated with specific desensitization at the Out-Patient Department of the Rigshospital in Copenhagen, and it has been considered important only to desensitize with the supposed specific allergen or allergens, or with the one of several which gave greatest intradermal reaction, and not to try nonspecific treatment. The material must therefore be considerd usable for a judgment of the value of specific desensitization.

In order to get information about the effect of the treatment, a follow-up examination of the treated asthmatics was started in December 1946, the aim of the examination being to include all asthmatic patients who have been treated at the clinic in the years 1943-45, thus establishing a period of observation of at least 3 years duration.

The patients have been summoned to the clinic to a control examination, and it is the intention later on to seek out in their homes those who did not turn up. On this occasion I shall state the results of the follow-up examination of the first 100 patients about whom we have succeeded in obtaining reliable information.

To a certain degree the material has thus been selected, consisting as it does of the first 92 patients who appeared in answer to the summons, and two physicians, who have answered in writing; moreover information about 6 persons who have died. The great majority of the cases mentioned in the following are therefore townspeople of Copenhagen.

As objective criteria of the condition of the patients are few and unreliable in periods without attacks, questioning has been the main line of action taken. Moreover, weight and vital-capacity have been controlled, and as for cases not bettered or aggravated, X-ray control has been carried out to the greatest extent possible.

Regarding sex and age the examined cases are distributed as follows.

TABLE I

Sex and age of the 100 re-examined patients.

	≤ 15 years old	> 15 years old	total	
female	5	43	48	100
male	10	42	52	

Further classification has been made on the basis of the diagnoses made after the first thorough examination on the first visit

TABLE II

Diagnostic classification of the 100 cases.

	female	male
diagnoses: asthma bronch.	29	26
asthma bronch. emphys. pulm.	5	4
asthma bronch. bronchitis chr.	6	10
asthma bronch. emphys. pulm. bronchitis chr.	8	12
	48	52

to the clinic (Table II). As will be seen from Table II, 55 patients suffered from pure bronchial asthma while 45 cases suffered from asthma + complications; furthermore two patients had bronchiectasis. None showed symptoms of heart disease.

A great many patients suffered from other allergic conditions, vasomotor rhinitis being a specially frequent complication, but in this statement regard has only been taken to the asthma, which in all cases was the main diagnosis.

The patients have been questioned as to known cases of allergic diseases in their families; of 95 patients, allergic heredity was found in 66 (67 per cent.) often with several cases in the same family. Among his patients *Bray* (1) finds 68 per cent, but in most statements the figures are somewhat lower (40-60 per cent). If we consider allergic affections in the antecedence only, these are dispersed as Table III shows.

TABLE III

Allergic heredity in 95 patients suffering from bronchial asthma.

Information about 95 patients	mother's family	father's family
allergic eczema	7	1
urticaria	4	1
vasomotor rhinitis	3	2
hay fever	4	0
oedema Quincke	0	0
bronchial asthma	30	14
migraine	7	1
	55	19

The great preponderance of asthma will be noticed, as well as the great difference as to frequency of allergic affections on the paternal and maternal side.

Of the 100 examined patients 91 have been treated with desensitization, whereas 9 have not been treated.

With anamnesis and intradermal tests as the basis the patients have been desensitized with extracts of the following substances (Table IV).

TABLE IV

Allergens in 100 patients suffering from bronchial asthma.

	Female	Male
Dust (house or factory)	24	27
Feather	16	12
Pollen	5	9
Flour	1	4
Horse dander	2	0
Cockroaches	3	0
Fungi	2	0
Rabbit's hair	1	0
Cat's hair	1	1
Orris-root	1	0
Shrimps	1	2
Mixed animal danders	0	0
Moths	1	0
Wool	0	1
	58	57

Most patients have been treated with one extract only, some with two and a very few with three extracts.

The patients have been asked if they continuously or in periods have been in contact with the substance or substances against which they have been desensitized, and if they have noticed that the substances provoke attacks (Table V).

TABLE V

	Yes	Probably not.
Contact?	92	4
Does the substance provoke attacks?	42	50

53 patients have had disagreeable reactions one or several times. (None have had allergic shock and only one patient, who got most violent attacks of asthma after the injections, gave up the treatment for this reason.)

TABLE VI

Disagreeable reactions in 91 patients treated with specific desensitization.

	Female	Male	
Disagreeable local reactions	18	14	32
Abscess	1	0	1
Urticaria	1	0	1
Vasomotor rhinitis	1	0	1
Allergic shock	0	0	0
Attacks of asthma	12	6	18
			53

In order to obtain a proper estimation of the results of the treatment the patients have been divided into the following groups.

1. Symptom-free.
2. Almost symptom-free (i.e. recovered to such a degree that the patient does not feel restrained by his asthma).
3. Bettered (i.e. pronounced recovery in the condition since first visit).
4. No change.
5. Aggravated.
6. Dead.

To get an impression of the degree of accordance of the patients' information with the actual facts, I questioned them about their consumption of remedies on their first visit and at the control examination. From Table VII it will be seen

TABLE VII

Survey over the patients' sonsumption of medicine after desensitization.

Consumprion of medicine	Reduced	Unchanged or *never medicine*	Increased
Without symptoms	9	2	
Almost without symptoms	10		
Bettered	16	12	
No change	2	15	6
Aggravated		2	5

TABLE VIII

Therapeutic result of specific desensitization of 91 asthma patients.

5 of the patients treated have died during the 3 years in question, one died from scarlet fever, one was shot during the German occupation, and three have died in status asthmaticus.

	Without symptoms	Almost without symptoms	Bettered	No change	Aggravated	Dead
Just after treatment	6 (6.6 %)	8 (8.8 %)	53 (58.2 %)	21 (23.1 %)	3 (3.3 %)	
3 years after	11. (12 %)	11 (12 %)	32 (35 %)	24 (26 %)	8 (9 %)	5 (6 %)

that there is good accordance between the patients' information about the clinical result and their consumption of medicine.

The results of the treatment are shown in Table VIII, where the first line indicates the condition just after the first series and the second line the condition at the control examination. It is seen that 73.6 % showed a remarkable turn for the better immediately after the specific desensitization and that the improvement of 59 % lasted for at least three years. However, part of the patients who at the control examination are classified as free of symptoms, almost free of symptoms or bettered are of opinion that the treatment was quite without effect and that they have recovered after that time; the following circumstances must

TABLE IX

15 patients who state improvement after desensitization, but who ascribe the improvement to other factors than the treatment.

	Without symptoms	Almost without sympt.	Bettered	
Untraceable cause more than 6 months after treatment	1		2	
After treatment by a chiropractor	1			
Changed occupation	3	1		
Change of dwelling	1		2	
Avoidance of the allergens	1		2	
Special asthma medicine	1			
Total	8	1	6	i.e. 16 %

be supposed to be the cause of the recovery which has taken place (Table IX).

When looking at the final result of the specific desensitization we therefore have to reduce the improvement after 3 years from 59 % to 43 % as being the real number of patients who may be supposed to have improved by specific desensitization alone. In comparison *E. Bruun* (2) in 1945 reported improvement in 87 % of 239 desensitizised asthma patients with at least 6-12 months' observation-time, and *Flensborg* (3) and *Salén* (4) report a similar percentage of improvement in asthmatic children.

Among the unchanged or aggravated patients 3 have after the treatment been granted invalidity pension; 6 others have had to neglect their work to such a degree that they have not been able to carry it out satisfactorily, or they have had to engage assistance. None of the flour workers of the present material, namely 3 bakers, a millworker, and a woman biscuitmaker, have been able to work again without serious discomfort.

SUMMARY

The author describes the results of a follow-up examination of 100 patients suffering from bronchial asthma. The patients were three years earlier treated by specific desensitization. After a lapse of three years 59 % still show a remarkable improvement, but 16 % of these claim other factors than the desensitization to be the cause of improvement; thus the final result seems to be an improvement in 43 %.

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ON ATYPICAL ASTHMA

By

GÖSTA BECKER,
Helsingfors.

Diagnosing bronchial asthma is usually easy. The typical attacks of dyspnoea with wheezing or whistling expiration are so characteristic that the disease can often be diagnosed with much certainty already on the background of the history of the case.

I propose to point out, however, that the concept of bronchial asthma also comprises conditions in which the typical paroxysms are absent.

The following examples will illustrate a type of asthma in which one would not think of this affection at first.

An eighteen year old intelligent and sprightly girl goes out to stay with relations in the country. She cannot sleep, though she feels tired and sleepy. She has a disagreeable, oppressive sensation in the chest, but feels easier when she sits up. During daytime, too, she is oppressed. The people think she is odd, the doctor diagnoses hysteria. The condition lasts for some years, then come typical attacks of asthma, from which she suffers for the rest of her life.

A thirty-five year old spinster complains of a heavy feeling in the chest. There are no typical paroxysms, no cough, merely an oppression "as if my chest were too tight." She is employed at a baker's, where the customers come with their own raw materials for bread and cakes. She is inclined to agree that the weight on her chest is heavier when she transfers the flour from customers' bags into a container. She is positive that there is no wheezing or whistling from the lungs, and in the course of repeated examinations I was unable to find any, although she came for examination

direct from work and the distance to my surgery was only a few hundred metres. Eosinophilia in the blood strengthened my suspicion of asthma, and a positive skin test for wheat flour established the diagnosis.

A thirty year old newspaper editor had himself got the idea that he perhaps had asthma. He had read a popular description of the disease. When he had to remain for some time at meetings where the air in the room became bad, he began to be nervous. There was no actual dyspnoea, and no wheezing or cough either, but he "felt distressed." Bromide and luminal, prescribed for him by his doctor, gave no relief. At the examination he seemed somewhat labile, lungs and heart occasioned no comment, but the blood showed 11 % eosinophile cells—without worm eggs in faeces. Skin tests were positive to several different extracts. Treatment with potassium iodide and ephedrine over a long period gave complete relief.

In the first case the sensation of oppression in the chest without typical asthmatic paroxysms for two years or so represented the preliminary stage of common asthma. What the position is in the other two cases I cannot say, as the period of observation is too short. It is possible that this is a special form of asthma, though this is a question of more theoretical interest.

The feeling of oppression in the chest is described differently by the patients from one case to another. Some feel that the chest is too tight, or as if there were a band around it; others feel a want of fresh air, and others again become anxious, and many in fact insist that they are "troubled beyond words," which at first suggests nervous trouble.

For the differential diagnosis one must have one's attention on lung and heart affections—especially incipient nocturnal heart-stasis trouble, and also hypertony, thyreotoxicosis and blood diseases. But if such affections can be excluded, one should be cautious about classifying people with a "heaviness in the chest" as simply nervous. Suspicion should be directed towards asthma. Eosinophilia in the blood strengthens that suspicion, and a skin test may give an etiological diagnosis.

However, the diagnosis will not be clear unless one thinks of

asthma. I believe that nervousness once again, as so often before, must surrender territory to an organic disease.

Another form of asthma is also easy to overlook.

At any rate here in Finland the diagnosis of chronic bronchitis is disappearing more and more from the hospital case reports. No doubt this is partly because our possibilities of diagnosing cardiac defects with chronic stasis, pulmonal tumours and other lung diseases are improving, but to a great extent the reason is that these cases of chronic bronchitis so often are asthmatic.

In most cases a careful inquiry into the history will reveal typical asthma paroxysms, but by no means always. The patient has had "only a cough," or irritation with or without expectoration.

The cough or the irritation come often when it is cold out of doors or when the patient from cold air comes into warmth, when he is at the theatre or cinema. It is often so tormenting that the patient must leave in order not to attrract attention or disturb others. The usual cough medicines have little effect.

For many years my neighbour in the medical faculty was a colleague who especially in winter began to cough as soon as he came into the meeting room, and he continued to cough till the end of the meeting. No cough medicine did any good. His mother had a similar cough; indeed, others in the family were said to have suffered from a cough with the same "clang." Unfortunately I did not get his cough analysed. He had no asthmatic paroxysms. But I wonder if my diagnosis was not right after all?

As a rule the physical examination reveals emphysema with or without bronchitic symptoms; the sedimentation rate is normal or high, there may or may not be expectoration, but if there is, it is usual to find eosinophile cells in it. Blood eosinophilia is usual, but sometimes absent. Skin tests generally give polyvalent reactions. But treatment with iodine and adrenalin, or sulphur and sulpha-preparations, nearly always results in marked improvement, after the patient has taken cough medicines of various kinds for years, or even stayed at a sanatorium.

I am well aware that what I have described in the foregoing is nothing new to this gathering. But I believe that allergologists

should accept the view that asthmatic paroxysms are not necessarily part of the picture of bronchial asthma; that cases with only "heaviness in the chest" may just as well be asthma as "chronic cough" may. These patients do not go to the specialists at first, but consult the general practitioners. If practitioners are to have their attention directed to this matter, they must be informed from competent quarters.

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ON THE BEARING OF ALLERGY ON THE TREATMENT OF CERTAIN GASTRO-INTESTINAL DISEASES, ESPECIALLY ULCER

By

O. SANDERS OLESEN

(Aalborg)

A case in my practice about ten years ago led to my making the present investigation. I shall briefly summarize the history of the patient.

A man, nearing the end of the fifties, with a long-standing disease of typically ulcer-character (typical pains, periodicity and repeated haemorrhage). The usual medical cures failed, and then the patient was operated after some severe haemorrhages. Nothing was found. Later on, however, when being tested for allergy there was a strong positive cutaneous reaction to egg, and a strict eggless diet brought about complete freedom from symptoms. A single relapse later on was due to breaking away from the eggless diet.

As a result, I made a routine examination for allergy by intracutaneous tests on my gastritis and ulcer patients and dyspeptics who, through their anamnesis, were open to suspicion of being associated with allergy.

It is well known that the gastro-intestinal tract may be the seat of allergic reaction (acute or chronic), but I considered that the question of how great a role allergy played in well-defined affections like ulcer and gastritis demanded closer examination. I shall present the results of my investigations in the period 1941-46 (see table II).

In that period I examined 279 patients with roentgenologically demonstrable or otherwise clinically definite ulcer, 53 patients

with demonstrable gastritis changes, and 32 patients with dyspepsia in whom no ulcer or gastritis was found but for whom the medical history suggested allergy.

Positive skin reactions were found in 212 patients = 58 % of the total material:

141 reactions in gastric ulcer patients (50 %)
39 reactions in gastritis patients (75 %)
32 reactions in Group III (100 %).

The allergens found were (see Table I).

TABLE I

Allergen	No. of reactions
egg	60 = 26 %
milk	68 = 30 %
meat	0
fish (plaice)	1 = ½ %
rye	44 = 14 %
wheat	14 = 6 %
oats	20 = 9 %
greens	3 = 1½ %
potatoes	10 = 4½ %
tomatoes	3 = 1½ %
carrots	4 = 2 %
apples	1 = ½ %
barley	3 = 1½ %

More uncommon foods have been omitted, as they would only cause isolated acute allergic reactions in conjunction with their consumption but, on account of their rare occurrence, would scarcely be of etiological significance in chronic gastro-intestinal diseases. Therefore I tested only with foodstuffs ordinarily appearing in the daily diet.

Patients without a positive reaction are then treated in the usual manner (diet, medicine, etc. as may be suitable for their case, and operation where necessary).

Patients with a positive reaction were experimentally placed

TABLE II

	Gastric Ulcer 279	Gastritis 53	Dyspepsia 32	Total 364
+ reaction	141 = 50 %	39 = 73 %	32 = 100 %	212 = 58 %

on a diet from which the allergen demonstrated was removed completely; otherwise the diet was as complete as possible.

The results of this treatment were then followed up by controlling the patients, partly by personal conversations, partly by sending them questionnaires to fill up, with the results shown in Table III.

TABLE III

	Ulcer 141	Gastritis 39	Dyspepsia 32	Total 212
Cured	31 = 22 %	14 = 36 %	25 = 78 %	70 = 33 %
Improved	57 = 40.5 %	13 = 33 %	4 = 12½ %	74 = 35 %
Unchanged	53 = 37½ %	12 = 31 %	3 = 9½ %	68 = 32 %

Of ulcer patients

31	apparently cured completely	22 %
57	definitely better	40½ %
53	unchanged and have had to abandon this treatment	37½ %

Of gastric patients

14 = 36 % cured
13 = 33 % improved
12 = 31 % unchanged

Group III

25 = 78 % cured
4 = 12½ % improved
3 = 9½ % unchanged

In the great majority of cases these records are for an observation period of over two years; moreover, in most cases they were patients with very severe, disagreeable symptoms and

frequent relapses and who had gone through thorough medicinal cures previously, with no particular result.

The necessary conclusion to be drawn is that it would be advisable as a matter of routine in the case of patients coming for examination for gastro-intestinal affections to test them for allergy to foodstuffs; there is perhaps a 50 % chance of discovering such an allergy and thereby of relieving or freeing the patient of his complaint in a simple manner.

The material shows that a third of the patients have improved, i.e. have become much more fit for work and have much fewer symptoms, but without being wholly well. In these cases there is no doubt that allergy plays a coordinate role, so that treatment for it will only relieve the patient of some of the discomforts, unless he has to be operated; the question then arising is: does the allergy disappear because he is operated? It is scarcely likely.

In my material there are a few previously operated patients who only became symptom-free after the removal of the allergen.

Another aspect of the matter is that it would be of advantage to have a more sure method for demonstrating allergy; I have no doubt that some of the patients who have been placed to another group owing to absence of reaction must nevertheless have had some form of allergy to a foodstuff, and that, the demonstration having failed, they have missed the chance of being helped to get rid of their complaint.

DISCUSSION: C. JUHLIN-DANNFELT

C. Juhlin-Dannfelt: The results which doctor Olesen here has stated must without a doubt be considered as fairly remarkable. At the usual routine intradermal skin tests of ulcus ventricular cases with standard series of allergen extracts we have thus only in exceptional cases found obvious positive skin reactions for nutritive allergens. Analogous results have also been obtained at most of the investigations about these matters, published earlier. Therefore, I should like to ask if it has been verified by skin tests on non-allergic persons with the extracts that doctor Olesen has used that these extracts have given a clearly negative result on *non-allergic* skin.

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A CONTRIBUTION TO THE STUDY OF MAN's REACTION TO INSECT BITES

(*Preliminary report*)

By

BJØRN HEILESEN

The subject, an adult man, who has never been exposed to bites of Aëdes aegypti (a mosquito) is bitten by this insect 5 times a day. During the first 8 days the bites do not give rise to any subjective or objective reactions. On the 9th day delayed reactions are seen at the sites of bites which are 24-48 hours old. On the 15th day new bites cause immediate reactions with wheals and erythema. These immediate reactions subsided after an hour, but about 24 hours later delayed reactions developed in the same places. In continued experiments the immediate reaction increased in size and the incubation period of the delayed reaction was diminished.

These observations may indicate that the subject in the experiment has become sensitized to the substances secreted in the skin by the mosquitoes in biting. Attempts to demonstrate the presence of antibodies in the blood as given by *Prausnitz-Küstner*, by means of extracts of the salivary glands of the mosquitoes or "whole" mosquitoes, gave a negative result.

A more detailed report on these experiments and others performed later is being prepared.

(*The Transactions of the Congress will be continued in Fasc. III*).

REPORT ON THE FOURTH MEETING OF THE ACADEMY OF ALLERGY

ST. LOUIS, MO., U.S.A.,

DECEMBER 15th, 16th, and 17th, 1947

BY

JOSEF LIŠKA

Prague

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REPORT ON THE FOURTH MEETING OF THE ACADEMY OF ALLERGY, ST. LOUIS, MO., U.S.A., December 15th, 16th, and 17th, 1947.

BY

JOSEF LIŠKA,
Prague.

An audience of about 400 highly interested and interesting men and women gathered in the Hotel Jefferson, St. Louis, Mo., on December 14th, 1947, to attend lectures and view the scientific as well as the commercial expositions.

The lectures, followed by extensive debates, as a rule took all day, and the evenings were devoted to corresponding colloquia concerning seven main topics, each presented by one chosen referee. The lectures were presided over by outstanding experts, and each topic had, besides its author, a special leader of discussions. The subject matters of the lectures were diverse and varied a great deal. Four lectures were delivered in memoriam: Dr. W. W. Duke, Dr. Aaron Brown, Dr. J. Alexander Clarke, Jr., and Dr. Warren T. Vaughan.

For better orientation, let me list excerpts from the lectures:

The Induction of Asthma-like Attacks in Subjects with "Idiopathic" Asthma: Francis C. Lowell and Irving W. Schiller, Boston, Mass.

A group of subjects with "idiopathic" bronchial asthma were exposed to aerosolized allergenic extracts as well as aerosolized solutions of histamine and acetyl-beta-methyl choline. Measurements of the vital capacity and ink tracings of the respiratory curve were made before and at intervals after exposure to the allergen or drug. Note was also made of signs in the chest or subjective sensations of asthma.

In general, pulmonary reactions did not follow inhalation of allergenic extracts in this group of subjects. These results differed markedly from those obtained in a previous study of subjects with asthma of the "extrinsic" type. With respect to the response to inhaled histamine and acetyl-beta-methyl choline and the modification of this response by certain drugs, the group of subjects with "idiopathic" asthma reacted in a manner similar to those with "extrinsic" asthma.

Clinical Aspects of Climatotherapy for Allergic Diseases. F. B. Schutzbank, Tucson, Arizona.

A general discussion concerning the value of climatotherapy for allergic diseases. The author then relates the possible psychosomatic influences on patients that have been relieved by a change of climate, and discusses cases where credit is given to a change of climate, but is actually due to an elimination of an offending antigen. Furthermore, the type of case that can be helped by a change of climate is discussed by the author, and he also includes the beneficial effect of climate on each complication of bronchial asthma as emphysema and bronchiectasis.

The Reversing Influence of Low Humidity on Intractable Asthma. O. E. Egbert, El Paso, Texas.

When cases of severe asthma with much bronchial mucus transfer from humid atmospheres to an arid one, they go into reverse in forty-eight to seventy-two hours and become so dry that the necessary treatment is to stimulate mucus flow. Bronchial casts are rapidly cleaned out and are not again a problem; in fact casts are not seen in asthma of residents of an arid climate.

This reversal is by no means the answer to the asthma problem. The old problem is relieved, but new ones are created that seem quite as baffling as the first. In the text dry cases are reported as serious problems. Authors are cited who show mucus as the worst problem of asthma, some opinions being that it is the fundamental cause of intractable asthma. In this paper the

author reported severe dry asthma following moist asthma, seeming to disprove mucus as the fundamental cause.

However, the reversing action does have its advantages in many cases. Emphasis is put on reversing the treatment to meet the reversed symptoms.

Growth Patterns of Allergic Children. Milton B. Cohen and Lewis E. Abram, Cleveland, Ohio.

The regular recording of height, weight and age on the Wetzel grid affords a simple and reliable method of determining body types and the details of growth and development of children. The authors demonstrate the use of the grid and present the analysis of the records of allergic children showing: 1) the distribution of allergic children in the various body types, as compared with the distribution of these types in the general child population; 2) the deleterious effect of active allergy on growth and development: 3) the beneficial effect of control of the allergy on the patterns of growth and development.

Pulmonary Function Studies in Bronchial Asthma. Louis Tuft, George I. Blumstein and V. M. Heck, Philadelphia, Pennsylvania.

In the study of bronchial asthma, insufficient attention has been paid to physiology and to pathologic physiology. The present report deals only with external respiration in the cases of allergic disease; that is, the transport and interchange of gases between the outside world and the blood, as determined by vital capacity, minute volume respiration, ventilatory equivalent, maximal breathing capacity and breathing reserve. The latter terms are defined and normal values presented for each of the phases. The results of pulmonary function studies on a group of asthmatics with and without emphysema, before and after the administration of a broncho-dilator, are presented. Studies on a group of hay fever patients before and during the pollen season are also discussed. The possible clinical applications of the procedure are enumerated.

Adjuvant Treatment of Hay Fever with Emulsions of Pollen Extract, Falba and Mineral Oil. Mary Hewitt Loveless, New York, N.Y.

Thirty-three ragweed sensitive patients, who have been under intensive "booster" therapy and immunological observation in previous years, were given adjuvant mixtures containing pollen extract, falba and mineral oil in volumes averaging 0.3 c.c. Three cases took one injection containing from 200 to 650 protein nitrogen units. Twelve others received a total dose of 1,000 units and one patient 2,000 units in a single injection. With the exception of one man who was given 11,000 units in 4 treatments, all remaining cases were administered from 1,200 to 7,250 in two visits.

The immune response of these subjects was judged by means of threshold conjunctival tests and, in some cases, also by serological titrations of the passive transfer type. The clinical response was appraised by a combination of the patient's impression and a daily record of his hay fever hours. Comparisons were then made between the patient's response to adjuvant therapy and to the author's booster treatment given in earlier years.

Although final conclusions must await the end of the pollinating season, preliminary observations suggest that the adjuvant therapy is as efficacious as the multiple injection treatment with aqueous antigen.

Studies on the Mechanism of Dermatitis Venenata in the Guinea Pig. Seymour B. Crepea and Robert A. Cooke, New York, N.Y.

A 5 % extract of poison ivy in alcohol or Mazola oil was applied to the depilated skin of thirty albino guinea pigs, three times each week. All animals became sensitive in ten to fourteen days. Reactions were read in twenty-four and forty-eight hours. The animals were anesthetized, bled from the heart, and the spleen removed. A suspension of spleen cells in normal saline was made.

An area of the depilated skin of a normal guinea pig was patch tested with 5 % extract of poison ivy in alcohol. After 24 hours

the extract was removed with acetone. Negative reactions were obtained. The normal animals were then injected intraperitoneally with either 2 c.c. of serum, or the suspension of spleen cells or wash water, which had previously been saved for control experiments. The following day the animals were patch tested at a new site by applying 5 % poison ivy extract, and observations were made after twenty-four and forty eight hours. In six of ten tests guinea pigs injected with serum gave a positive reaction. Animals injected with spleen cells reacted in sixteen of nineteen trials, whereas in all but one case the wash water failed to transfer.

It is concluded that there is an antibody mechanism in the cells and in the serum which mediates the dermatitic reaction in guinea pigs made sensitive to poison ivy.

Corn Sugar as an Allergen. *Theron G. Randolph and Leona B. Yeager, Chicago, Ill.*

Corn ranks with wheat and milk as an important food causing chronic food allergy. Corn is in every way the most difficult food to avoid, since this entails elimination, not only of corn as such, but all products containing corn starch, corn oil and corn sugar.

In the majority of patients hypersensitive to corn, the ingestion of corn syrups and corn sugars will not only precipitate clinical symptoms, but cause a more prompt reaction than they do to other forms of corn. These reactions are likely to be missed, unless patients are correctly diagnosed and the usual sources of corn are eliminated from the diet prior to experimental feeding. Investigation reveals that corn starch is a common binder used in the preparation of tablets, and is frequently employed in the binder or surface-sizing of paper and cardboard used in the wrapping and packaging of foods.

Allergic Factors in Gout. *Joseph Harkawy, New York, N. Y.*

The paper presents a study of three patients with gout whose attacks were induced by sensitization to foods, pollens and infection. The first patient gave a history of attacks of gout that occurred during the early summer and fall. Ingestion of wheat in-

duced a moderate attack of gout with a rise in the uric acid. Ingestion of wheat plus the subcutaneous injection of timothy extract, resulted in swelling of the right knee and involvement of the ankle and foot, with a rise in the uric acid. Similar symptoms occurred following ingestion of wheat, plus a subcutaneous injection of ragweed.

The second patient had attacks of gout that were most marked in the spring and summer. The symptoms were often preceded by a cold in the head and wheezing respirations. The patient was found to be hypersensitive to various trees, to timothy grass and the ragweeds. Following an injection of a mixture of tree extracts, a gouty reaction occurred in the right knee and left ankle. The right knee also became involved after an injection of timothy extract.

The third patient had been subject to attacks of sinusitis and sneezing for twenty-five years. This patient was hypersensitive to dust, various trees, timothy grass, ragweed pollen and some foods. A marked reaction was obtained to milk. During the ragweed season, the patient had symptoms of hay fever and symptoms of an arthritis involving the right knee and right wrist, with an elevation of the uric acid. On one occasion, in the spring of the year, an attack of arthritis involving the right wrist, knee and neck occurred after the ingestion of milk. The author points out the significance of his findings, and a therapeutic approach to the various problems is discussed.

Histopathology of Atopic Dermatitis and of the Characteristic Atopic Reaction to Patch Test. Frank A. Simon, Louisville, Kentucky.

The natural lesions of atopic dermatitis and the cutaneous reactions to patch tests with human dander, on normal or on scarified skin, both show histological evidence of epidermal injury. The epidermal cells are swollen or entirely destroyed, their cytoplasm takes the pink eosin stain and their nuclei may be seen in various stages of lysis and pyknosis. Intercellular and intracellular vesicles may be seen in the epidermis in some sections. In

the reactions to patch tests some of these are associated with the openings of sweat glands, but this association is not found in the natural lesions. There is an infiltration of inflammatory cells in the dermis and also in the injured areas of the epidermis. The cells involved in the reaction and the pathological changes apparent in these cells are so much alike in both the natural and artificial lesions as to suggest that the fundamental pathology is the same in both.

Quantitative Immunologic Studies with Allergens. Stanley F. Hampton, Samuel C. Bukantz and Mary C. Johnson, St. Louis, Mo.

A freshly prepared low ragweed pollen extract was divided into two portions. Glycerin in a final concentration of 50 % was added to one. The plain and glycerinated extracts were then stored at room temperature, 6° C (ice box), —25° C (deep freeze) and —70° C (carbon dioxide box) for one year. These extracts were studied at intervals during this period for their phosphotungstic acid precipitable nitrogen content, their capacity to precipitate antiragweed rabbit serum and to neutralize skin sensitizing antibodies of human serum.

Glycerinated extracts retained their precipitating and serum neutralizing activities, whereas the plain extracts stored under the four conditions showed significant loss of activity. The phosphotungstic acid precipitable nitrogen, however, showed relatively little or no consistent reduction.

Freshly prepared plain and glycerinated extracts were heated to 56° C for thirty minutes and studied as described above. The heated plain extracts showed great loss of activity, although the heated extract containing glycerin did not.

Studies in Contact Dermatitis. The response of healed specific dermatitis sites to stimulation with another contactant. *Max Grolnick, Brooklyn, N. Y.*

The response of healed sites of contact dermatitis to the subsequent application of a second and unrelated allergenic extract

is reported. Reactions were elicited in 42 subjects who were sensitive to either krameria or poison ivy, but not to both. The reaction sites were allowed to heal. After an interval of 4 to 12 weeks, each site was retested with the other excitant. In most instances, control tests had also been made on uninvolved skin. A typical contact type of reaction was present at the sites which had been twice stimulated in 14 of the patients. In 10 of the paients on whom controls had been done, the sites were negative. The rule of non-specific stimulation of a specifically sensitive area, and the subject of local skin senstivity are discussed.

The Thermolability of Human Precipitin and Anaphylactin. Wm. *B. Sherman, R. A. Cooke, S. B. Crepea and L. M. Downing. New York, N. Y.*

The skin-sensitizing antibody differs from the blocking antibody and from most antibodies induced in experimental animals by its relative thermolability, being destroyed by heating the serum four hours at 56° C. It was therefore of interest to study the effect of heat under similar conditions on the antibodies induced in humans by artificial sensitization with horse serum. Serum obtained from patients during and after serum sickness showed antibodies with the properties of precipitin, skin-sensitizing antibody, and anaphylaction, producing passive sensitization of guinea pigs. After heating four hours at 56° C, the serum did not precipitate with the antigen, did not sensitize normal skin, and did not produce a passive Dale reaction in guinea pigs. The heated serum did not show evidence of a blocking antibody inhibiting the reaction of horse serum in skin tests on sensitive patients. In contrast to the human antibodies, the corresponding antibodies in the serum of rabbits artificially sensitized to horse serum were not inactivated by similar heating.

The thermolability appears to be characteristic of various human antibodies, rather than specifically of the skin-sensitizing antibody (reagin) og spontaneously sensitive human beings.

Personality Factors in Allergic Nasal Disorders as Determined by Analysis of Recorded History Interviews. John. H. Mitchell, Ch. A. Curran and Ruth N. Myers, Columbus, Ohio.

In order to evaluate the significance of personality factors in patients concerning their nasal symptoms, verbatim recordings of twenty-four initial history interviews were made in twelve cases of perennial vasomotor rhinitis, and twelve cases of allergic rhinitis, six of which were seasonal and six perennial. Children were not included. In obtaining histories, the non-directive interview technique was used. A careful analysis of each statement made by the patient gave statistical confirmation to a number of clinical impressions.

The patients were encouraged to talk freely about themselves. Direct questioning was avoided. Such histories, the authors believe, are of diagnostic value, and patients frequently during their second visits expressed noticeable improvement in their chief complaint and in many of the collateral complaints. The patients' statements were carefully evaluated. Those statements indicating feelings of confusion, conflict, hostility, rejection of self or by others, social maladjustment, escape, dependency, fear or unhappiness were scored as negative emotions.

The author concludes that the careful mathematical analysis of twenty-four recorded histories seemed to give further evidence of the value of the point of view which uses psychological as well as physical techniques in diagnosis and treatment.

Further Studies Concerning the Essential Fatty Acids and Eczema of Childhood. A. V. Stoesser, Minneapolis, Minn.

A preliminary report on the influence of soybean products, on the iodine number of the plasma lipids and the course of eczema was presented in 1945. It covered observations made on 17 cases of infantile eczema which were divided into 2 groups. Eleven had localized weeping lesions with an average iodine number of the total fatty acids of the plasma lipids of 71, and 6 appeared to have generalized dry areas with an average iodine number of 98. When both groups were fed a soybean preparation, the first had an

increase in the iodine number to 118, with some improvement in the skin manifestations, while the second only had a moderate rise to 108 with little or no change in the skin condition.

The investigation has been expanded so that now there are complete studies on 90 cases of eczema of infancy and early childhood and 40 control subjects, representing about 300 determinations of the iodine numbers of the plasma lipids. All the children received at some time during the period of observation a soybean milk preparation containing 4 % soybean oil with an iodine absorption value ranging from 119 to 135. Those cases which did not respond satisfactorily were given pure soybean oil, either in addition to the milk or alone in fairly large amounts. The results are interesting. The children with localized moist erythematous lesions who apparently belong to the allergic or atopic type had marked rises in the unsaturated fatty acids of the plasma lipids, a situation which enhances the effectiveness of good general and local forms of therapy. The cases with widespread dry scaly areas of the seborrheic or ichthyotic types had moderate increases in the unsaturated fatty acids. All of the children of the latter group were not benefited.

Allergy in Japan. Ralph I. Alford, New York, N. Y.

The paper is an account of pertinent and interesting aspects of allergy as observed by the author in Japan. The sources of information consisted of personal interviews with Japanese physicians, and visits to medical schools, libraries and hospitals. Over forty medical books, reprints and reviews were interpreted and studied, with the help of a Japanese physician. There are many differences in environment in Japan which have influence on allergic disorders. Of the 260,000 square miles in Japan, only 15 % is under cultivation. Fifty % of the area is made up of forests containing such trees as oak, ash, birch, elm and poplar. The remaining 35 % of the total area is mountainous. Of the 15 % which is under cultivation, about 50 % is in rice fields.

The Japanese diet is markedly different from that in the U.S. Less milk, wheat, eggs and chocolate are consumed. House dust,

as it is known in the United States, is not a factor in producing hypersensitivity, for those substances which help to make up the house dust of an average American home are unknown factors in Japanese domiciles. There is very little hay fever in Japan, due to the absence of ragweed and plantain. Timothy grass is very scarce.

Cases of asthma that have been reported are, for the most part, due to molds, foods, animal dander, plants, bacteria and drugs. Some cases are reported from silkworm cocoons and horse meat. Urticaria is common, and many case reports and articles on this subject were found. The foods causing urticaria most frequently were meats, lobster, crab, mackerel, tuna and bonita.

The method of desensitization used by Japanese physicians is discussed. Very weak extracts are used. Non-specific protein methods of treatment are employed more frequently than specific desensitization.

Studies of the Cantharidin Blisters in Normal and Allergic Individuals. Max Samter, Chicago, Ill.

The differential count of cantharidin blister fluid in humans has been studied in a variety of pathological conditions, e.g. during the course of infectious diseases. Analysis of this literature seems to indicate that the local barrier between vascular layer and epithelium plays an important part in determining the character of the cells emigrating into the blister.

The present study examines the conditions which cause the presence of eosinophils in cantharidin blisters in normal and allergic persons. It attempts to clarify the behaviour of these cells in actively and passively sensitized tissue.

Poison Ivy: A Summary of One-Hundred Cases Treated with Aqueous (Alum Precipitated Pyrinide) Extract. G. E. Gaillard, White Plains, N. Y.

This paper reviews the methods employed in the specific treatment of poison ivy dermatitis and indicates the advantages ob-

tained from the use of an alum precipitated aqueous poison ivy extract.

A summary is given of results of treatment of one hundred cases, 50 % of which have been treated for a two year period and 25 % of which have been patch tested comparatively with aqueous and alcoholic poison ivy extracts.

The interest shown in the colloquia was unusually great, all debates were very lively and sometimes ended as late as midnight. The topics discussed were:
The Broad Aspects of Pathology in Allergic Conditions,
Drugs Used in Allergic Conditions,
Dietary Problems in Pediatric Allergy,
Management of Bronchial Asthma in Children,
Medical Management of Bronchial Asthma,
Allergy to Drugs, Endocrine Products, and Serum,
Management of Infective Asthma.

The scientific exhibition had several divisions; they were:
Council on Aeroallergens of the American Academy of Allergy;
Pollen Committee;
Mold Committee;
Committee on Education of the American Academy of Allergy;
Antihistaminic Drugs;
Histamine Antagonists in Allergy;
Intrinsic Asthma;
Dermatitis of Hands, localization;
Poison Ivy;
Bronchial Asthma in Infants and Children;
Studies with Steam-Generated Aerosols;
The Characteristics of Asthma in Infancy.

Commercial exhibits comprised: Pharmaceutical industry, Cosmetic industry, Production of Allergens, Drugs, Cosmetics, Inhalators, Air Cleaners, Manufacture of Hypo-Allergenic Bedding Products, as well as exhibits of various publishers, a Coca-Cola Bar and a "Camels" stand.

Scientific topics were preceded by Club topics, which were gone into at great detail and length, and had the undivided attention of all those present. New Officials were elected, as follows:

President CARL FIGLEY
Vicepresident WALTER WINKENWENDER
Secretary THEOD. SQUIER
Treasurer HORACE S. BALDWIN

The Congress ended in a typically American, informal way, by the chairman simply announcing: "That's all"—and everybody said Good Bye in the friendliest spirit, aware of the fact that something of lasting value had been accomplished.

Dr. *Joseph Liška,*
Prague.

Acta allergologica, 1948, I, 237—252.

From the Medical Out-Patients' Department of Rigshospitalet, Allergy Consultation, Copenhagen. Director: Professor Eggert Moller, M.D.

PREPARATION AND STANDARDIZATION OF ALLERGEN EXTRACTS[1]

By

EGON BRUUN

The reason why the Committee of the Northern Society has desired to bring on a discussion of this subject is the hope of obtaining suggestions of a better method of preparation than that which is used at present. In the Northern countries we ought at any rate to come to an agreement with regard to a *uniform* preparation of the extracts.

It would, perhaps, be more practical, if the manufacture of extracts were centralised in one single laboratory, which would subsequently carry out the distribution of the extracts to the different countries. As long, however, as the Scandinavian Customs-Union is a Utopian notion, this will probably have to be given up on account of difficulties of exchange alone. Still, it ought not to be impossible to obtain just as good results, if the different laboratories and clinics would work with a uniform method and, notably, if the laboratories could agree with regard to an exchange of preparations and extracts in order to get into contact with one another.

To begin with I shall account for our method of preparing the extracts in Copenhagen, and try to point out the advantages and imperfections of the method. The Danish extracts are prepared by the chemist *Barfod*, who started this preparation already in the nineteen-twenties. *Barfod* originally worked according to the method of preparing extracts suggested in 1916-17

[1] Opening address to the Congress on Sep. 10th, 1948.

by *Wodehouse* (45, 46) and *Wodehouse & Ohmsted* (47, 48). That method was subsequently modified and is now as follows:

(1) Preparation of the starting-material.
(2) Shaking of the starting-material in a slightly alkaline liquid.
(3) Filtration through sterile filters.
(4) Sterile bottling of the extract in sterile ampoules, and control of the sterility.

The Starting-Material.

Collection: The principle of collecting allergen-containing material ought to be that it is used *as far as possible in the condition met with in Nature.* It will be impossible to mention all those allergens which may come into consideration. With regard to them I refer to the excellent appendices to be found in several American textbooks (13, 28, 38, 39, 43, 44) treating of the subject, but with regard to some of the most important groups of allergens I shall report certain practical observations.

House dust can be gathered directly from the vacuum-cleaner or be collected with a brush from furniture, beds, and floors. Irrelevant substances, such as small stones or coal particles, are removed before the preparation of the extracts.

Hairs and danders of animals are the most easily obtained by currying the respective animal. This method is better than mere cutting of the animal, for by combing or currying some epidermal substance will always be removed together with the hair, and afford more active extracts.

Feathers can be plucked out of the bird or taken from the feather-bed used. In Denmark we have made the experience that this latter method is to be preferred, because feathers from "asthma feather-beds" have proved to yield good extracts, whereas new, freshly elaborated and cleansed feathers generally yield weaker extracts. That is probably due to the feathers in old feather-beds being infected with micro-organisms.—*St. v. Leeuwen*'s demonstration of *Aspergillus fumigatus* in the feathers of certain asthmatics' feather-beds are wellknown; anyhow it must be stressed that the best feather extracts are obtained either by directly plucking the birds and not cleansing the feathers (in Denmark we make good extracts from so-called Greenland feathers, which are unchanged feathers from different eider ducks, puffins and jackdaws), or by using feathers from the patients' feather-beds and pillows. Fresh, cleansed feathers are not so appropriate.

Fungi are collected by placing nutrient substances in the suspicious places; after a very brief time of exposure, the agar culture-medium will be overgrown with micro-organisms. Of them pure cultures are made, which are used for the preparation of extracts. In that way both the fungi and their spores are obtained. If a pure spore preparation is desired, the fungi must be cultivated until maturity and be shaken in a particular manner for spores, which subsequently are used for the preparation of extracts. This is a rather difficult method; nevertheless, *Flensborg* & *Barfod* (20) have succeeded in producing good spore preparations.

Pollen can be collected in different ways; generally the flowers are cut late in the afternoon, and the stalks are placed in vases filled with water. The vases must have oblique walls so that the flowers incline over the table top. The room must have been dusted beforehand and be protected against draughts. On the table top, under the vases, is placed a large sheet of white paper, and the flowers are shaken every day, the pollen being gathered from the paper.

Grasses need not be placed in water, but merely be put on the paper in small heaps. Most of the pollen species are shaken off in the early morning hours at sunrise.

The *pollen* of tree blossoms is collected on large sheets of white paper placed on the lawn under the trees, whose branches are shaken every day during the blossoming time.

Aliments. Milk is taken fresh from the cows that are on fodder without addition of soy or cottonseed cake.

Meat must contain as little blood as possible, and fat and connective tissue is removed before use. Both for meat, fish and milk it holds that it must be raw and quite fresh. Milk preparations are made from both raw and boiled milk, however.

Fruits and vegetables are procured fresh during the respective seasons, and the same applies to *cereals* and to *nuts*, which must be ripe.

Tobacco leaves must have been dried in the sun, and good preparations can be obtained *ad modum* Harkavy (21).

The simplest sensitivity test is examination with the respective allergens in an unchanged condition. The first skin reactions are known to have been performed by a little of the suspected substance being placed in a scratch on the skin, the principle always being that the material is as unchanged as possible; on the other hand, certain procedures, such as removing fat, are mostly necessary for the obtention of applicable preparations. In the preparation of extracts, two methods have asserted themselves. Some investigators, *e.g. Jaffé* (22), fear that some of the

allergenic properties are destroyed by preparation. Hence they caution against too vigorous endeavours to remove fat, colouring matter and the like. Others (23, 24) strive after purifying the extracts as much as possible by different procedures, and they do not even shrink back from boiling them, although it is known that certain allergens are changed by boiling, for example milk and the white of hens' eggs; house dust seems, however, to tolerate autoclave sterilisation (17), whereas the character of pollen is rather labile (3, 4). Moreover, too much "denaturalization" may result in false, irritative reactions, and damage the allergens. In Denmark the extracts are prepared according to the first suggestion. To begin with the starting-material is treated quite roughly, in some cases not at all. Then the material is pulverized in a mortar, and the fat is removed. This procedure may be performed with ether, carbon tetrachloride, toluol or acetone. Most of the allergens with the exception of certain fruits and vegetables must be freed from fat.

The pulverized, fat-extracted starting material is dried, and in this form it is applicable as allergen for scratch tests, but it should be kept in mind that, at this stage, it is a rather impure raw product.

From the above it is evident that the starting material in Denmark always is dry material, as we, out of consideration for the durability, deem it important that the preparation is in a dry condition.

Shaking in slightly alkaline liquid. By transforming the dry preparation into extract, a higher degree of purity can be obtained. This is feasible by (a) precipitation, (b) adsorption (29), (c) dialysis, (d) ultrafiltration (26, 36, 37), (e) ultracentrifugation, and (f) electrophoresis (1, 2), which will be discussed later.

The first aqueous extract was made by *Noon* (31) in 1911. He shook pollen in distilled water, and after freezing and thawing the extract was filtered and sterilized by boiling. *Lowdermilk* (27) used a sodium chloride solution instead of water, and *Coca* (11) replaced this by a slightly alkaline liquid with an addition of phenol. This Coca liquid is still used very much, but it has the disadvantage of decomposing by being boiled. Evans (18) has

modified Coca's liquid so that it can stand boiling, but he still adds phenol to it for sterilization.

Several authors have tried to substitute glycerine or alcohol for phenol, but according to our experience both glycerine and alcohol give rise to such severe local irritation that we will not use them. When the extracts after filtration are tested for sterility, the addition of phenol should be superfluous, and for fear of eventual hypersensibility for phenol, we have decided not to add either phenol or other chemical sterilizing substances. As far as I know, it is only in Denmark that neither phenol nor other sterilizing substances are added, and it should at once be said that in the allergy clinic of the Rigshospital where there are given about 500 injections a week, we have, as yet, only had one single case of infection, an insignificant abscess. We therefore regard it as being warrantable to omit adding phenol, if we have insured sterility.

As precipitant solution we use a slightly alkaline liquid consisting of

25 gm. of sodium chloride,
12.5 gm. of sodium bicarbonate,
distilled water to 5,000 gm.

1 gm. of the dry preparation is added to 100 ml of the solvent, and the mixture is shaken for 24 hours, and afterwards it is sterilized through sterile Seitz or porcelain filters.

Filtration. It is difficult to decide what filter is the most appropriate. What is decisive is *that the filter insures sterility at the same time as it permits the passage of the allergen, at least to a certain degree.* Both Seitz filters and glass filters come up to this demand. However, during World War II, it was very difficult to produce a sufficiently great number of these filters. We have therefore tried filtration through very thick paper filters. On re-examination of the patients it was found that the paper-filtered extracts were much more active than those filtered through china and asbest, but the sterility, as we had feared, was not reliable, whence paper filtration had to be given up.

After filtration, some extracts present a very strong colour,

and contain certain irritating substances, which can be removed by dialysis, however. The American College of Allergists has in 1945 edited a Manual on extract procedures, in which the most appropriate apparatus for dialysing is assumed to be a special cellophane tube called No. 600. This tube retains the larger protein molecules with the active principles contained in them, but permits of passage of electrolytes and low-molecular protein substances.

Sterility. After filtration and perhaps dialysis, the extract under sterile precautions is transferred to sterile ampoules, samples at the same time being taken for control of sterility. These samples must comprise cultivation both on aerobic and anaerobic substrates, and any one who prepares extracts of house dust, hairs of animals and feathers must regard this as an imperative duty. The word tetanus must stand as a warning that lack of precaution and conscientiousness in this respect may result in death.

Brewer (7) has suggested a thioglycerolate substrate which is very appropriate for both aerobic and anaerobic cultivation, and which is to be recommended, because it is so easy to handle.

The preparation of extracts proper, as it is done in Denmark, is thus achieved, and it should be emphasized that, on the whole, we are working with good and appropriate preparations.

I have had the opportunity of comparing Danish extracts both with English (*Park, Davis* & Co.) and American extracts (*Lederle*), and I daresay that the Danish extracts in by far the majority of cases were just as good as the Anglo-Saxon ones, moreover being cheaper, whence we always make use of Danish preparations, even though we might desire improvements in a few points. This holds particularly for fruit and vegetable preparations, for in a few cases I have observed that the Danish preparations were ineffective, whilst the English and American preparations afforded positive reactions.

When I tried to get to the bottom of the question, I found that we in Denmark always work with standard solutions of 1 : 1000,

whereas, in U.S.A. and, partially, in Sweden too, are used allergens of articles of food in concentrations of 1 : 100 or 1 : 10, and in case of very juicy fruits, for instance water-melon and grape-fruit, even in the proportion 1 : 1. It is probable that in future we have to use more concentrated preparations of articles of food.

If it is desirable in more complicated cases to "purify" the allergens still more, there are, as was previously mentioned, several possibilities (precipitation, adsorption, dialysis, ultracentrifugation, ultrafiltration, and electrophoresis). *Clowes* (10), as early as in 1913, tried to precipitate an active fraction of an aqueous pollen solution with acetone, and other authors (5, 8) have later endeavoured to prepare a highly active fraction of aqueous allergen solutions by precipitation, while *Sutherland* (41, 42) in 1942 and 1945 alleged to have prepared a very active house dust extract by adsorption of house dust extract to benzoic acid and several subsequent procedures.

Feinberg (19) has suggested a precipitation method which is reported to be particularly applicable for meat and fruit extracts, and epidermic substances. The method consists of extracting the raw material with 1/20 or 1/50 n NaOH, after which is added HCl to maximum precipitation of sediment. The sediment is dried and used both as dry preparation and for solution. Here it is thus a question of an isoelectric precipitation, it being taken for granted that the allergens have an isoelectric point, thus being ampholytes[1]. *Feinberg*'s name and experience vouch for the quality of these preparations; personally I have no experience with preparations prepared in that way, and I may, perhaps, be permitted to say that the procedure seems to me to be a little inadequate to the purpose as long as it is not known whether the allergen has the same isoelectric point as the chief quantity of the protein substances, and for diagnostic purposes it is indeed to be preferred that as much as possible of the extractive substances is contained in the preparations.

[1] An ampholyte is a substance which has the character of both acid and base; the hydrogen ion concentration at which these two characters are equally pronounced, is called the isoelectric point.

The last-named procedures (ultracentrifugation, ultrafiltration, and electrophoresis) are of a more special character and are notably known from the chemistry of protein substances. They require special apparatuses, and as we in Denmark at present dispose of only one ultracentrifuge and very few electrophoresis apparatuses, we are for the time being quite unable to make use of these methods on a large scale. In Sweden, which is indeed at the head of these methods of research, there should on the other hand be a possibility of rational examinations of the value of these methods.

Standardization.

There does not exist any plain and accurate method for measuring the potentiality of the allergen extracts (4, 30). The best procedure would be isolation of the active fraction in chemically pure condition, and preparation of standard extracts of it on the base of weight or volume. However, a protein allergen in a pure, homogenous condition does not exist, and it is known with certainty that certain allergens, for example pollen, contain several, chemically different allergens. These facts complicate the whole problem, because a completely pure preparation from a clinical viewponit may, perhaps, not be desirable at all, and will at any rate render standardization difficult.

The first attempt at standardization was made by *Noon* (31) by introducing the Noon unit, for pollen extracts, which means the amount of allergen contained in one microgram of phleum pollen. *Noon*'s efforts are commendable, and the Noon-pollen-unit is still used in many places, but it has later been shown that the activity of different kinds of pollen varies with the seasons, and with certain botanical and climatic conditions. Other authors (14, 40) presumed that the protein was the active allergic principle in pollen, and they standardized their extracts starting from the nitrogen content by precipitation with phosphotungstic acid, but *Newell* (30) in 1942 showed that phosphotungstic acid also precipitates some allergically inactive substances, such as ammonium compounds, and basic amino-acids. Of all the methods based on determination of the protein content may be said

that, as long as it is not known with certainty that the allergen is identical with the protein content of a substance, and only with this, they will be unsuccessful, and indeed much seems to indicate that the active substances in certain cases are not proteins (6), but that they may be polysaccharides.

A few authors (9) have tried to use fixation of the complement as a form of standardization, but the method had to be abandoned when the results proved not to correspond to the allergic activity of the allergens. Some researchers finally work with molar units (33) and with velopan units, but none of these methods has been successful.

The Danish preparations are standardized purely percentually so that the extract which is obtained by addition of 1 gm. of the starting-material to 100 gm. of dissolving fluid, is called 1 per cent, and the other concentrations are obtained by simple dilution. By multiplication with a simple fraction this standardization of pollen can easily be converted into Noon-pollen-units.

It is obvious that this standardizing method is not satisfactory. The circumstance that no regard is paid to the varying solubility of the allergens alone in reality renders this standardization illusory. We have tried to remedy this by performing nitrogen determinations and by determinations of specific gravity, but neither of these procedures afforded a correlation between the results found and the allergenic properties of the substances.

The only method which seems to be quite adequate, is that developed and applied by *Cooke* & co-workers (14, 16) who make use of Prausnitz-Küstner's principle. This standardization consists in determining the smallest quantity of extract required for *in vitro* neutralization of a constant amount of serum from an allergic. It is evident that this method for mere practical reasons has its limitation, and besides, on account of the prevailing hepatitis epidemic, we have during recent years been very cautious in performing Prausnitz-Küstner experiments in Denmark.

Salén (35) makes use of a *biological* standardization without applying the Prausnitz-Küstner principle by testing his extracts on allergics, and standardizing them, starting from the magnitude of the reaction on an allergic whose power of reaction is known

to him, controlling at the same time that the extract does not give reaction on non-allergic persons. This method affords excellent results, but it requires constant disposal of allergic and normal persons who are willing to submit to these experiments.

In the Allergy clinic of the Rigshospital we have been of opinion that we might on an empirical basis put the limit at a concentration of 1 : 10,000, for we always demand positive reaction to 1 : 10,000 before we speak of allergy. Positive reactions only in the higher concentrations *may* be due to allergy, but they are often unspecific. In dubious cases we must resort to Prausnitz-Küstner's experiment. We are well aware that by imposing such severe demands as positive reaction to 1 : 10,000 we sometimes miss a diagnosis, but on the whole I think that the criterion is fairly adequate in practice. Anyhow, numerous control examinations have never given positive reactions in the normal (*i.e.* patients without allergic diseases and *without allergic disposition*) with extracts in a concentration of 1 : 10,000.

A solution proper of the problem of standardization I am unfortunately unable to suggest. Cooke & co-workers' method and the procedure applied by Salén are commendable, but they both require large allergological departments and materials of allergic and non-allergic patients in order to be applied.

The Durability of the Extracts.

This little account of preparation and standardization of extracts cannot be terminated without a brief mention of the durability of the extracts. By being stored at + 4 ° C., the majority of the Danish extracts are applicable, *i.e.* without demonstrable reduction of their allergenic activity, for at least a twelvemonth. This only holds for inhalation allergens, however. Extracts of vegetables show reduced activity already after lapses of from 3 to 6 months, and a few extracts, for example certain fruit extracts, must be prepared fresh every time they are to be used. We have introduced the rule that all *standard* preparations without exception are renewed every three months, and all fruit extracts are prepared afresh for every patient. The standard preparations

are used for diagnostic purposes only. On the other hand, the auto-housedust extracts prepared for treatment can be used for up to 1 year. We think that this procedure affords the greatest possible safety for both patients and ourselves.

It is obvious that this account must be superficial in many respects, but then my intention has been merely to point out the problems. It is the first time that Northern allergologists are assembled for a discussion of common problems, and our close geographical neighbourhood, our common language, and many other circumstances tend to facilitate co-operation. I hope that on the ground of this lecture and subsequent discussion we may come to an agreement with regard to uniform preparation and standardization of extracts in the Scandinavian countries. That will make our scientific work and clinical results more comparable than they are today.

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DISCUSSION: P. H. NEXMAND, H. MALMROS, R. NORRLIND, B. ARNER, O. MIDTTUN, P. BONNEVIE

Nexmand, P. H.: I was very much pleased to hear Dr. Bruun emphasizing the importance of using raw materials for cutaneous tests or, at any rate, preparations which have been made according to as simple methods as possible. Whereas preparation and standardization of the extracts out of regard for the

desensitization of the patients with asthma and hay fever is a very essential problem for allergologists, the problem is much less important for dermatologists, because it is very rare that desensitization has a convincing effect on skin diseases. In dermatology the preparations are preferably used as a diagnostic adjuvant. In allergic dermatological research it is necessary to work with as pure experimental conditions as possible, and as long as we are unable to obtain the pure allergen, it will be useful to leave it to the allergic organism itself to select the efficient factor contained in the substance we shall examine. The great importance of the experimental conditions I observed in testing patients with Besnier's prurigo with human dander. After intradermal injection of extract of human dander, prepared according to the method suggested by Keller, I in contradistinction to the Freiburg Clinic found unspecific positive reactions both in allergics and in normal persons. On electrophoresis with *raw* human dander suspended in a 0.9 % sodium chloride solution, a characteristic papulous reaction (*Simon*'s atopic reaction), however, developed in the patients with Besnier's prurigo whereas the normal failed to react.

Malmros, H.: The majority of allergists probably agree as to the preparation of allergen extracts requiring procedures which will not injure the allergen itself. In case of bacterial allergens, it is somewhat different, however. It is possible that better preparations are obtained, if the bacteria are triturated or the capsule is removed.

As regards the question of the biological examination of different allergen extracts, it ought to be possible to solve it practically through collaboration between the Northern countries. We ought to exchange not only extracts which we regard as being good, but even bad extracts in order to have them tested somewhere else. Then it may, perhaps, be found that the preparation is not so bad as it seemed to be, but that it gives distinctly positive reaction when tested on a patient who is really hypersensitive to the substance at hand. The biological test is important, but it is time-consuming, and it may be rather difficult to obtain appropriate cases in which the test can be made. If collaboration should be established, it ought not to be very difficult to collect those fifty extracts which usually are tested for the standardization, and to

test them on patients with sure allergy. It will not be strictly necessary that the patients who are submitted to the tests, are staying in a hospital.

Norrlind, R.: When testing I have used a bacterial allergen the bacteria of which have been broken up by freezing down to —15° C. and shaking them together with sterilized steelballs in a vibrator for about 8 hours at ordinary room temperature. With this bacterial allergen only immediate wheal reactions were obtained. The patients who showed positive tests, also showed positive tests to other protein allergens in a greater frequency than those who showed negative tests to bacterial allergen. Passive transfer according to Prausnitz-Küstner's method generally was not a success. (See Norrlind: Prurigo Besnier, Acta Dermat.-Ven., Suppl. XIII, 1946, Stockholm.)

Arner, B.: The question of bacterial extracts and how they should be prepared, which Dr. Malmros has broached, is very interesting. Since we have no access any more to the bacterial extract from the Sächsische Serumwerke (Germany), we have in Södersjukhuset no opportunity of making any experiments with bacterial allergens. This bacterial extract is reported to have given direct reactions, and Norrlind is reported to have obtained such even with the bacterial extract prepared by himself. We have tried a great number of autovaccies without, however, observing a direct reaction in any case. On the other hand, we have observed several uncertain tardy reactions. Norrlind's extract is reported to have been prepared through maceration of the bacteria, by which the bacterial albumin is dissolved. According to Feinberg, cutaneous tests with such extracts of macerated bacteria have not produced direct reactions in America. In several quarters it is opined that the molecules of the bacterial albumin are too small to be able to act as genuine allergens, but that they should be a kind of haptenes. Perhaps, the polysaccharides of the bacteria do act as allergens.

It would be of great value, if a substitute for the old German bacterial extracts could be tested so that we might possibly get a little nearer the solution of the question of bacterial allergy, which at any rate in Sweden plays a great rôle.

I have carried out an experiment with a highly concentrated

bacterial extract, but the result was not very encouraging. With α-streptococci from a patient with chronic bronchitis I have with Dr. Nordin's help in the State Bacterial Laboratory produced a strong concentrated extract of bacteria desiccated in cold and subsequently ground in cold. This extract produced fairly strong local reactions on the patient as well as on myself and, at the same time, a certain general reaction with fever, the whole reaction probably being due to a toxic action of the extract.

Midttun, O.: With regard to the question of a possible central laboratory for the Scandinavian countries for the preparation of allergen extracts, I shall draw attention to the difference in the articles of food used in the different countries, and the consequent difference in allergy of diet.

A Swedish fish extract contains small herrings only living in the Baltic and unknown for example in Norway. The same holds for collective extracts of vegetables.

As regards extracts of articles of food, I therefore think it will be better that each country prepares its own extracts on the base of the articles of food which are used, and which empirically oftenest give allergic reactions.

Dr. Malmros asserts that no allergen has been found in a pure state. I shall draw attention to Tiselius and Erickson from P.P.D. tuberculin having prepared a substance the molecular weight of which they know—and this substance gives "immediate reaction" in tuberculous guinea-pigs.

I should like to ask the Swedes, if they carry out investigations in electrophoresis of bacterial vaccine in order to get at the bacterial allergy, if possible.

Bonnevie, P.: The standardization ought to be based on the determination of that concentration of extract which does not cause any toxic reaction, cf. the choice of test substance concentrations in eczema patch tests after determination of that concentration which, under the testing conditions, is the threshold value for toxic irritative reaction. Just as there are criteria which separate caustic and eczematous dermatitis reactions, there ought also to be set up criteria distinguishing between toxic and allergic urtica reactions. I agree with Dr. Nexmand in what he said about it;

it throws light on the principal demands which I have set up in my introduction.

The biological standardization requires very much work, which we might conveniently divide among us, and we should thus also be enabled to work with reliably comparable preparations here in Scandinavia. This implies uniform preparation and a uniform method of standardization of extracts. As a practical result of this first congress of Northern allergists I therefore propose electing a common committee for the fixation of methods and the distribution of work between us.

According to Dr. Bonnevie's proposal it was agreed to elect a Northern committee on common terms of reference for the preparation and standardization of extracts in the Scandinavian countries. As members of the committee were elected E. Linko, Z. Erickson-Lihr (Finland), O. Wilander, C. Juhlin-Dannfelt (Sweden), O. Andrup, Sv. Dirk Henriksen (Norway), P. C. Barfod, and E. Bruun (Denmark).

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From the Pension Board's Hospital, Åre, Sweden. Chief: V. Hedström.

NERVOUS CONDITIONS ACCOMPANYING ALLERGIC DISEASES

By

V. HEDSTRÖM

Nervous conditions accompanying organic diseases are but little known, and yet, the existing psychic touch in the organic diseases is of great importance. In every disease as well as in case of accident, a psychic factor must be reckoned with, and also convalescence is always accompanied by a psychic influence. The reactions within the same group of constitution or personality are fairly uniform provided that the external conditions under which the patients live, are more or less identical. Both in brief and long-standing morbid conditions, the fairly uniform reactions of a previously psychically healthy person mostly depend on the intensity of the disease and the disturbances it causes in the patient's relation to his (her) surroundings. In case of chronic diseases a gradually increasing fixed depressive neurasthenic touch in the morbid picture is observed. There are a great many contributory causes, such as the patient's individual mode of reacting within the possibilities he has in continuing to render life as tolerable as possible for himself and his surroundings. In persons who at the onset of the disease are not psychically healthy or up to the mark, the reactions become different. A psychasthenic reacts in his own way, an obsessional neurotic in his, a schizoid in his way, etc.

It is always of great importance in what way the disease influences the patient's life. A person who, before the onset of the disease or the accident, has the greatest difficulty in managing his affairs, reacts in another way and more strongly than does a

man who, at the onset of it, sees some way of arranging things. Economically dependent persons often react in a manner greatly reminding of pension neurosis, similar conditions, however, also being observed under other circumstances. Morose conditions due to compulsion of any kind whatever, readily exacerbate the morbid symptoms and have a tendency to fix them. Lacking social adaptability, for example difficulties in performing the work on account of the reduction of working-power, conflicts in the working-house, long-standing matrimonial discord, etc., are contributory factors. Unfortunately the treatment of such patients is not easy, because the complaints frequently are rather fixed.

If one, after this general summarizing orientation concerning the relation between psychic morbid conditions and organic diseases, focusses the allergic disorders, a great many morbid symptoms are found which, in my opinion, may be either of an allergic nature or of a neurasthenic type. In a great many allergic conditions a general reduction of force prevails. Sometimes it presents itself as a transitory state of tiredness, which sometimes degenerates into faintness; sometimes a more constant state of tiredness is observed such as presents itself in a severely psychasthenic patient.

In psychiatry it is known that shock due to allergy may induce depression and conditions of confusion. It may be of interest to mention that allergenic therapeutics may entail nervous discomforts. Thus *Morginson* and *Joachimson* and others have already reported cases of psychic distress after penicillin treatment, for example nervousness, depression, euphoria, and conditions of confusion. In case of periodically recurring psychosis, there is good reason to search for possible allergic conditions, particularly whether there are allergic diseases in the patient's family or whether he has previously suffered from some allergic disease or other. *Clark* also thinks that periodical conditions of psychosis may be of allergic genesis. He reports moreover that disturbances of sleep—sleeplessness or the reverse—may be entailed by an allergic condition, and that in case of dietary allergy they may yield to an adequate diet. In a great many periodical conditions of fatigue, particularly such as occur at a definite moment of the day, I have been able to discover an absolute relation with dietary

allergy. Headache, even of a type other than migraine, is likewise reported as a possible allergic symptom (*Rowe, Sulzberger* and *Baer*). *Brown* and *Goitein* emphasize that hypochondriasis is a symptom generally found in allergics. The common term hypochondriasis in my opinion is not adequate in this connection. The great majority of dietary allergics—at any rate the aged ones—have symptoms resembling gastritis, and the gastro-intestinal disturbances of which they complain should in my opinion be called changed body sensations similar to all the diffuse sensations which allergics frequently complain of having here and there in the body. To the afore-named discomforts, namely, tiredness, headache, and disturbance of the body sensations, above all from the gastro-intestinal canal, here also belong frequently occurring cardiac disorders, such as palpitation, sometimes extrasystoles, and paroxysmal tachycardia (*Sulzberger* and *Baer*). On examination these patients prove to be hypersensitive to many substances, above all to articles of food. These discomforts, which are often incomprehensible to the patient, and which may entail changes of vital importance to the allergic, secondarily give rise to great discomfort. He seeks after help, consulting physician after physician, if he does not experience any improvement. One must understand this migration, for one certainly has not to deal with persons taking refuge behind their illness. Secondarily to the discomfort and the reduction of his working-powers, and because both he and his surroundings ignore the cause of his disease, he incurs all sorts of inferiority complexes. He becomes pensive, thinking of his future and of his family. If he has not suffered from sleeplessness before, he incurs it now. Consequently he experiences headaches and further reduction of his working-powers. He frequently suffers from anxiety and restlessness. His nervousness increases and he is quite disabled. In my opinion, a great number of allergics who have not heretofore received the adequate treatment at disposal, are classified with the large group of patients termed neurotics. Here is a question of quite a large percentage of the previously so-called neurosis patients. Many of these patients are not psychiasthenics, as might be assumed after a superficial observation, but rather allergics, often, perhaps, latent

allergics with flaring up allergic symptoms and secondary neurasthenia.

It was formerly assumed that bronchial asthma primarily was of psychogenic origin. In our day there are still some researchers who advocate this conception. Before the doctrine of allergy was developed during the time from 1900 to 1930, that conception was more or less generally adhered to. As I shall show later, the psychic traumata which can give rise to attacks of asthma, are secondarily conditioned. Bronchial asthma, just as the great majority of llergic conditions, is a chronic affection with very great variations of intensity. The working-power is reduced considerably. Just as in other allergic conditions, though in a still higher degree in bronchial asthma, the patients is diffident owing to the rapid changes and the incalculability of the manifestations, continuing to ruminate over his mystic illness. His diffidence increases. He does not dare to or will not stay anywhere, he does not dare to eat anything, and he puts all sorts of outer circumstances into relation with his affection, and, as a rule, justly. Even during intervals free from discomfort the patient is, perhaps, more easily fatigued than other allergics, and the hazard of infection in the upper air passages and the lungs is distinctly greater than in nonallergics. Cold, fog, wind, and strong heat as well as bodily exertions are very hard to bear. Thus one has to deal with a condition of chronic diffidence. The severest symptoms mostly occurring at night—the horizontal posture exerting an unfavourable influence on lungs and heart—the patient's sleep is disturbed. During an attack of asthma he cannot sleep at all, and the rest he needs so much is not obtained. The meals are mostly anticipated with some aversion, because eating generally enhances the asthmatic discomfort. During an attack of asthma a repast is quite out of the question. Consequently, the patient's general condition exacerbates, and the physical as well as the psychic resources of strength decrease. In addition the allergic reactions together with the attacks of asthma have a tendency to increase. All this entails a state of agitation which often increase to anxiety, particularly in patients with long-standing or intense asthmatic discomfort. The anxiety may occur primarily on account of acid hunger due to pulmonal as well as

cardiac exertion and insufficiency. Chronic cases now and again present anxiety of cardial type. It must, however, be emphasized that cardiac anxiety has a strong psychogenic touch. The acute warning symptoms, which resemble dread, are due to severe affective conditions. When the patient experiences anxiety, he anticipates severer discomfort and, consequently, becomes still more agitated. Dread and anxiety in asthmatics increase with age on account of the associations becoming more and more mechanical. Dread and anxiety gradually occur the more readily after slight injuries as the patient immediately anticipates an attack of asthma. The actual neurosis finally induces the conditioned reflex. Attacks of asthma induced by different kinds of emotion are the result of such as it were auto-suggestive reflexes.

In bronchial asthma the symptoms change relatively rapidly as is also observed in affections of several other organs or organic systems such as the cardiovascular system, the gastro-intestinal, and the musculo-articular system. In such morbid conditions with rapidly changing symptoms the psychic influences are of great importance, sometimes quite dominating the picture, and acting as causative factors for the occurrence of the disease. Insignificant organic disturbances may instinctively or consciously give rise to distinct symptoms through psychic responses. These organic systems indeed have a possibility of reacting easily and rapidly.

In Sweden symptoms of pension neurosis do not manifest themselves so much in bronchial asthma as in other chronic diseases. For during an attack of asthma or in case of slight asthmatic distress, the patient's physical strength is so essentially reduced that the question of invalidity need not be discussed or examined. On the other hand, it often happens that such sociologico-medical viewpoints aquire actuality during longer symptomless or fairly symptomless intervals of latent, semilatent or suballergic conditions, which are met with for example in that category of individuals whom I have discussed before, namely the large group of persons requiring assistance who suffer from tiredness, now and again from diffuse gastric discomfort, a sensation of heaviness in the head or headache, disturbances of sleep without understandable

cause, cardiac discomfort, all of them symptoms which may behave as if they were of purely primarily nervous origin. I have had the opportunity of observing this in a great many cases of tiredness. The symptoms of tiredness are often due to psychic causes or they are manifestations of internal medical diseases of a non-allergic type, but attention should be focussed on the latent and semilatent conditions, for the sensation of tiredness which distresses the allergics fuses with and gradually is aggravated by conditions of tiredness of psychic origin. It has been alleged that asthma patients in a psychic respect should be endowed with an inferior constitution, a characteristic feature being that they are very labile. As children they should be whimpering—but then, what children are not so when they are ill—as well as bright and lively, and the great majority of them on growing older could be classified with the group of psychoneuroses. Personally I have been unable to discover that asthma patients differ from other ordinary patients by anything but the peculiarities due to chronic morbid conditions. On examining the asthma patients treated in the mountain sanatorium of Åre during the time from January 1, 1943 to June 30, 1946, I found among 572 asthma patients 57 (*i.e.* 10 per cent.) with psychic disturbances so conspicuous that I deemed it warrantable to add to the chief diagnosis the diagnosis of the psychic morbid condition. Among those 57 persons were nine altogether who could not be grouped as suffering from neurasthenia due to asthma. It is a question of six men and three women in whom mental debility, psychopathy and schizophrenia, respectively, were diagnosed, and two cases of psychoneurosis in the male patients as well as psychopathy, and two cases of hysteria in the female patients. Thus it has not been possible to demonstrate a primary psychogenic cause of the asthma. In all the cases the primary condition was allergic. As far as I know, the psychic inducement of the asthmatic attacks is always secondary, but it may be so conspicuous that it dominates. In case of psychic inducement of asthmatic attacks the cause is usually an affective condition due to apathy. Asthmatic distress is often observed when the patient is depressed by something which has happened to him, he may have received bad news in a letter, he may have read

about a serious accident in the newspaper etc. Many times, perhaps mostly—particularly in chronic cases—are observed dread and apprehension of the occurrence of an asthmatic attack with subsequent anxiety especially in the evening when the patient is trying to go to rest or sleep. As I have mentioned before, one notices the actual neurosis with the conditioned reflex, and this is not remarkable. How shall a person he endowed psychically, who under the pressure of a chronic disease does not incur neurasthenia? All the psychogenic causes of the occurrence of asthmatic discomfort may be incurred by any asthmatic whatever, if the irritation and inducement are strong enough. In the genuine psychasthenic the irritant need not be so strong as in the psychically normal person.— —A particular type of neurasthenia in asthma patients is met with in those who for rather long periods are in the habit of using some form of iodine or other. It is remarkable that they are not more frequently met with. From 1943 to the first half of 1946 inclusively, I have among 572 asthmatics only met with two cases of hyperthyreosis, which may be due to iodine medication. In both patients the neurasthenic discomfort vanished after operation, the asthmatic condition in one of them apparently having vanished definitely. The nervous component in bronchial asthma in these cases—the hyperthyroid component —partially at least is provoked secondarily hormonally. In bronchial asthma (just as in urticaria) remarkably low values of metabolism are often observed without its being otherwise possible to perceive symptoms of hypothyreosis.

Hansen mentions that a relation between the asthmatic discomfort and the cyclothymic conditions may very often be found in asthmatics. Cases are on record in which the asthma disappeared during conditions of manic-depressive psychosis, and recurred during free intervals. This has been connected with a change of the ion concentration of the blood during the psychosis, which should influence the vegetative system and, hence, cause the asthmatic discomfort to subside. These periodical conditions of asthma in manic-depressive psychosis, however, seem to be very uncommon.

I have intentionally alluded to the psychic morbid conditions

in bronchial asthma, because the symptoms of this allergic disease behave so remarkably. Secondary psychic discomfort is likewise found in numerous other allergic diseases. They are not discussed separately in this paper, because the nervous disorders are similar to those observed in the most important group of allergics who have heretofore been classified with the neuroses, and whom I have described as suffering from diffuse discomfort, namely a sensation of tiredness, disturbances of body sensation, headache, and disturbances of sleep. If the conduct in life is regulated on the ground of this explanation, the neurasthenic discomforts generally disappear. The nervousness will sooner have to be characterized as a phenomenon of exhaustion, though often complicated by conflicts due to want of common sense on the part of kinsfolk, fellow workers, and even doctors through social difficulties arising because of the disease. As mentioned above, we certainly have not to deal with the patients seeking refuge in their disease. On the contrary, the frequent visits of doctors imply an exponent of inability to explain these obscure allergic conditions. I emphasize this also, if anybody should raise the objection that autosuggestion in this respect certainly is of importance. In my opinion this group, as mentioned above, ought to be separated from the psychasthenia or psychoneurosis group. Neurasthenia is the adequate expression of the nervous discomforts of these allergics.

It is generally known that a great number of neurosis patients are exposed to all sorts of decrepitudes. They easily incur affections of the upper air passages, because they are very susceptible to infections. These patients to a great extent belong to the large group of diseases which I have described, and they are these patients in whom the treatment of the nervous discomforts has afforded such good results through fever therapy with sulphur and albumin preparations (for example sulphosin and pyrifer), calcium medication and, why not, even insulin, which indeed in a way is desensitizing too.

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From the Medical Out-Patient's Department of Rigshospitalet, Allergy Consultation, Copenhagen. Director: Professor Eggert Moller, M.D.

ON THE EFFECT OF STAYS IN NORWAY ON DANISH ASTMATIC CHILDREN

By

EGON BRUUN and EBBE KJEMS

It is a long-established experience that asthmatics may improve, or become free from symptoms, with a change of climate. The very geographical prevalence of this disease, which occurs chiefly in low, damp coast-regions in the temperate zones, indicates that climatic and geological factors play a part in the manifestation of the disease.

Asthma is of rare occurrence among mountaineers (25), and Kämmerer (14) considers that the higher an asthmatic is placed, the better will he feel. However, that it cannot be the altitude in itself that is the decisive factor, appears from Hurst's statement (11, 12) that Johannesburg, which is at a very high altitude (5,740 feet above the sea), is decidedly an asthma locality, whereas Death Valley (California), which is below the level of the sea, is a suitable place for asthmatics to live in. Tiefensee's examinations (11, 12) of the regional distribution of asthma in East Prussia seem to indicate that the condition of the soil is of importance to asthma, and van Leeuwen (15) and Kämmerer (14) also mention that sandy soil and rocky ground—irrespective of the altitude—are better for asthmatics than clay and marsh soil.

In 1930-32 Burckhardt (3, 4) published a number of thorough investigations into the effect of mountain climate on asthma, having compared the condition of the patients with meteorological reports and the altitude, and arrived at the result that both altitude and atmospheric pressure are of minor importance, whereas

humidity (fog, rain, snow) and sudden changes of temperature tend to a high degree to release the paroxysms. Most interesting in this connection is the communication by Rappaport and collaborators (18) about seven patients with pollen asthma who, in a room free from pollen, developed asthma on a day with a great fall of the barometer and resulting change of weather. When dampness is so often stated as an asthma-releasing factor, it is possible that it is not the damp weather in itself that is the cause, but the barometric conditions determining he damp weather. Rappaport *et alii* mentioned that the patients with pollen asthma mentioned above were absolutely able to stand rainy weather *without* any fluctuations of the barometer, and Jochims (18) also arrived at the view that variations of humidity and temperature exert no influence, and Hulting (9, 10) considers that neither the barometric height nor dampness and fluctuations of temperature play any part at all. It cannot be denied, however, that a cold and damp climate favours the development of acute infections of the respiratory tract (6) and in certain cases such infections may accelerate or produce paroxysms; Tuft, therefore, considers that in particular the asthmatics who have *complications of an infectious nature* should be sent to another climate. Alexander (1) has the same view.

According to the statements given above, it seems as if the beneficial effect of a stay in a mountain climate is due more to the settled height of the barometer, with chiefly dry weather, prevailing in certain mountainous regions than to the altitude itself. To this must, however, be added another factor of great importance: the allergenic milieu in mountainous regions is quite different from that of damp lowlands; this applies especially to the fungous and bacterial flora vegetating in the lowlands, but also to the smaller proportion of dust per unit of air in mountainous regions. The fact that considerable results may be achieved solely by clearing the patient's environments of dust, appears from the value of van Leeuwen's chambers and from the very favourable results achieved at the Home for Asthmatic Children in Årsta (Stockholm) (10, 20, 21), but with the patient staying in a suitable mountain climate it should be possible to produce a treble

effect: (1) Environments that are more freed from allergens, (2) a settled barometric height, (3) relief from the complicating infections of the respiratory tracts.

In the autumn of 1945 the Danish Red Cross received from the Norwegian Red Cross an invitation to send asthmatic Danish children to Norway and in this manner it became possible for us to gain experience of the effect of a radical change of the environs of asthmatic children. Even if we cannot speak of an actual *mountain* climate, as the children were just placed at an altitude of about 600 feet above the sea, they were still accommodated in a dry inland climate in contrast with the damp insular climate to which they are accustomed in Denmark.

From the spring of 1946 a total of 220 Danish children have been staying for 3 to 4 months in Norway, namely 63 girls and 157 boys at ages from 8 to 12 years. The children were picked out from all over the country on the basis of a medical certificate which just had to state whether the child was suffering from asthma. But the gravity of the disease was not considered; the applications were complied with in the sequence in which they were received, so that mild, medium severe and severe cases were dealt with indiscriminately. The material has been classified on the basis of the anamneses into mild, medium severe and severe cases; by *mild cases* we understand such as have mild paroxysms less than once a month, *medium severe* cases comprise patients with paroxysms several times a month, or protracted periods several times a year, and *severe* cases are patients with paroxysms at least once a week, or with protracted paroxysms during which they are confined to bed at least once a month. When these criteria are employed, the material proves to comprise 28.4 per cent. mild cases, 35.1 per cent. medium severe, and 36.5 per cent. severe cases.

We have been in touch with 215 out of the 220 children, or 97.7 per cent. The after-examination took place in the course of the holidays months (from June to August 1947) when it was

difficult to come into contact with the children; we hope that we shall manage also to get hold of the remaining five, but there is no reason to believe that the missing 2.3 per cent. will be able to alter the results arrived at.

The *immediate* results, i.e. the condition of the children on leaving Norway, look brilliant (Table 1). No less than 97.7 per cent. showed considerable improvement when the homeward voyage was started.

TABLE 1

Condition of the children on leaving Norway.

Symptom-free	Improved	Unchanged	Worse	Total
138 =	72 =	3 =	2 =	215
64.2 %	33.5 %	1.4 %	0.9 %	
97.7 %				

The question to be answered now is *how the children manage after their return from Norway*, as the period of observation is now up to one year. We have therefore called up the children for after-examination, and in the case of children living outside Sjælland we have asked the parents to fill in a questionnaire requiring detailed information about the child's asthma three months after his or her return and six, nine, and twelve months after.

We examined 107 children ourselves, 108 cases being elucidated by means of questionnaires. The results will appear from Table 2.

Two things will appear from this table. First that, after the return, a displacement takes place from the group "freedom from symptoms" to "improvement". Many of the children who had no symptoms in Norway developed asthma again after their return, but the disease has changed its character so as to be less marked than before the stay in Norway. This reduces the group of symptomless patients and increases the group of the improved. Secondly, the table shows that the improvement remained constant all through the period of observation, as the groups symptomless +

TABLE 2.

Condition of the children 3, 6, 9, and 12 months after leaving Norway.

Time after stay in Norway	3 months		6 months		9 months		12 months	
Number of children.....	161		106		37		29	
Symptom-free	51=31.7 %	78.3 %	25=23.6 %	69.8 %	13=35.1 %	78.3 %	12=41.4 %	79.3 %
Improved	75=46.6 %		49=46.2 %		16=43.2 %		11=37.9 %	
Unchanged	31=19.3 %		27=25.5 %		6=16.2 %		5=17.2 %	
Worse	4= 2.5 %		5= 4.7 %		2= 5.4 %		1= 3.4 %	

improved were constantly from 70 to 80 per cent. In this table the duration of the period of observation has been considered, but it appears that, after the children have come home, the results remain unchanged for at least a year. When the total result is stated at the end of the period of observation, whether this has been 3, 6, 9 or 12 months, it appears as in Table 3:—

TABLE 3.

Result at the end of the period of observation.

Number of children	161
Symptom-free	43=26.7 %
Improved	82=50.9 %
Unchanged	33=20.5 %
Worse	3= 1.9 %

It will appear from Table 4 that the best results were achieved in the milder cases, whilst they were scarcely as good in the medium severe and still poorer in the severe cases.

TABLE 4.

Result in the different groups of degrees of asthma.

	Total	Mild cases	Medium severe	Severe
Symptom-free	43	23=53.5 %	13=20.0 %	7=16.3 %
Improved	82	15=18.3 %	30=36.6 %	37=45.1 %
Unchanged	33	3= 9.1 %	16=48.5 %	14=42.4 %
Worse	3	1	1	1
Total	161	42	60	59

DISCUSSION

In stating the total preliminary result, it must be said that the great majority of the children seem to have experienced a beneficial effect of their stay in Norway, also on the long view, and it must be stressed that no treatment whatever was administered to the children during their stay in Norway. A few authors, e.g.

Diener (11), point out that specific treatment should be given simultaneously with the climatic treatment; we should very much like to do so, but so far it has not been practicable.

The results obtained are just as good as Turban's (24) and Burckhardt's (3, 4) results from Davos, i.e. at a far higher altitude (1,500 m.), and are quite similar to the results of Brock (2) at Bad Dürrheim (710 m.). This leads us to the very important question of the altitude at which the patients should preferably be placed; as yet we do not know anything certain about this question, and our results at an altitude of 200 m. are not inferior to those obtained in Davos at an altitude of 1,500 m. The existing examinations seem to indicate that the two factors which are decisive to the effect of the climatic treatment are freedom from allergens and settled heights of the thermometer and the barometer (3, 13, 17). It will hardly be possible to fix the most suitable altitude at a definite number of metres; it is undoubtedly less important whether the patients are placed at altitudes of 400 or 800 metres so long as the air is as free from allergens as possible. The only thing that can be said with certainty is that the point is to place the patients above the fog-limit. In most places in Norway this is at an altitude of 300 m., so that the patients should be placed above this level. In other countries, such as Switzerland, higher altitudes should undoubtedly be preferred. At "Halmrast" in Norway, which is situated only about 200 m. above the sea, we had the experience that during the autumn months, when a great deal of fog is coming in from the Randsfjord, the children had certain "asthma days".

Consequently, we are of opinion that it should be endeavoured to find a place where the air is as free from allergens as possible, where the thermometric and barometric heights are settled and where there is no fog. When the patients are placed in such a climate, they have a chance of an improvement of long duration, at any rate in the case of children who have not yet developed emphysema. Bronchitis does not seem to prevent the attainment of a good result; it is quite peculiar to see the bronchitis of these children disappear very rapidly, as soon as they come to Norway.

Considering the results achieved, we are of opinion that we should continue our endeavours to send astmatic Danish children to Norway. We have been unable to elucidate the question as to how long the children should stay there. The literature on the subject states from 3 to 12 months as the most suitable; for practical reasons (school attendance) we have had to be content with 3-4 months, but it is possible that the lasting result might be improved by a still longer stay. We attempt to make up for this by keeping in touch with the children, sending them to Norway again in case they need it. In this manner we hope to be able to prevent disablement owing to emphysema and bronchiectasis, waiting for the *spontaneous recovery,* which occurs in about *40 per cent.* of the cases in children (5,8). It should be possible to accomplish this at least; on the basis of the results produced here, we consider it probable that a still higher percentage can be reached, but at present nothing can be said with certainty about this question.

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DISCUSSION: M. KOBRO, E. BRUUN

Kobro, M.: We have been very pleased in Norway to learn that the Danish asthmatic children recover so much from their disease after their stay in Norway. It is less well-known by us that the Swedes, too, have seen so good results of treatment of asthma in mountain air.

But from a therapeutical point of view the problem of asthma is not solved simply by moving the patients to higher altitudes. We, too, have seen our asthmatic patients being freed from symptoms when they leave their ordinary environments and go, say, to Lillehammer. But every day we see examples of the reverse. In the Norwegian mountain districts asthma is no rare disorder. Patients from these parts of the country often get rid of their symptoms when they go down to the lowlands, e.g. to Oslo. During the last few years I have been employed at a hospital situated at a high altitude outside Oslo. But I am not under the impression that our therapeutical results in asthma are better there than, for example, those obtained at the "Rikshospitalet" in the city.

When an asthmatic improves with a change of climate, three causative factors may be imagined.

First, the tendency to bronchitis may possibly be influenced by atmospheric conditions. A dry and pure atmosphere must be supposed to influence favourably the infectious factor of the disease. Patients with asthmatic bronchitis of this nature will do better in forest or mountain air than in urban environments or in a raw sea climate.

Secondly, a change of environments means that at the same time the asthmatic leaves his house allergens. The farmer from the mountains improves when he comes to town because he leaves his straw mattress, his barn and his domestic animals to which he has become sensitized and to which he reacts. The townsman

improves in the mountains because he leaves his special house dust.

Thirdly, the psychological, suggestive and educational factors play a material part. This applies in particular to asthmatic children, who are largely neurotics and who may for some time benefit from leaving the home circle and coming under different conditions and supervision by other people.

From these viewpoints we may doubtless take it for granted that it will hardly be special climatic health resorts or special altitudes which in themselves exert a curative effect on asthma. In the so-called climatic treatment of asthma we must consider the change of environment itself to be the most important.

We have thus a reasonable explanation of the fact that the Danish asthmatic children improve during their stays in Norway, where they will always be heartily welcomed.

Bruun, E.: I agree with Dr. Kobro that the change of environments plays a very essential part in climatotherapy; but still I also believe that the settled barometric height is of great importance. In the case of children I hardly attribute so much value to "the psychological, suggestive and educational factors" mentioned by Dr. Kobro. But the improvement of the children's general health, their recovery from bronchitis, their possibility of going in for sports etc. are of extremely great importance, and these boons can only be obtained by means of change of climate. In Denmark it is intended to establish a home for asthmatics at Hobro (Jutland) and, even if any initiative to help these children must be approved of, I believe it would be better to send the children to a suitable place in Norway—partly because Hobro has the same unsettled meteorological conditions as the rest of Denmark, and partly because Hobro does not offer the same possibilities of winter sports as Norway.

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From the Medical Out-patient Department of the Rigshospital, Copenhagen. Allergic Consultation. Chief: Professor Eggert Møller, M.D.

BLOOD EXAMINATIONS IN HAY FEVER PATIENTS DURING ACUTE ALLERGIC SHOCK

By

THOMAS CASTBERG and MICHAEL SCHWARTZ

Allergic shock in man comes unexpectedly in most cases, or at any rate under conditions prohibiting exact examination of the composition of the blood while the shock lasts; as a result, our knowledge of any changes there may be in the state of the blood during allergic shock rests upon a relatively slender foundation.

In animals, anaphylactic shock is generally considered to be analogous to allergic shock in man; the pathogenesis is assumed to be the same in both reactions: an antigen-antibody reaction liberates a histamine-like substance which thereupon induces the local and general symptoms (4, 6). It should be observed, however, that in anaphylactic shock the symptoms may vary considerably from one kind of animal to another, just as in man the allergic shock has no uniform symptomatology. Finally, the liberation of histamine (or H-substance) will not suffice as an explanation of all symptoms, and it is assumed that other humoral intermediaries (heparine, acetylcholine) are also active.

In *anaphylactic shock* (in animals) there occurs a sudden change in the permeability of the capillaries and in the tone of the smooth muscles in various organs; this rapidly involves changes in the blood pressure, respiration and the chemical and cytological composition of the blood.

For example, there may be a greater or smaller fall in the blood pressure (7), haemoconcentration, together with a decrease in the number of leucocytes and thrombocytes (5, 6, 8, 9, 12, 20, 21, 22, 28, 29), and the coagulation time is prolonged (up to

several hours) (21, 27). Increase of the blood sugar has been observed (15), as well as a higher non-protein nitrogen in the blood (12), acidosis (1, 10), and often a reduced body temperature (3).

None of these changes is constant, and it would seem that they depend more on the duration of the shock than on its intensity. Finally, it is maintained (Moon (14), Joltrain (11)) that none of these changes can be said to be specifically characteristic of anaphylactic shock, as they may be observed in shock of any other etiology.

ALLERGIC SHOCK IN MAN

The pathology of anaphylactic shock has been fairly well investigated, but this cannot be said of human allergic shock. Nevertheless, so many observations have been accumulating that all pathological findings in animal anaphylactic shock have now been recognized in acute general allergic reactions in man (18).

In 1914 Widal et al. (29) set up the concept of "haemoclastic crisis" or "colloidoclastic shock", manifested by lowered blood pressure, leucopenia, haemoconcentration, decrease of the blood coagulation time, and a fall in the refractometric index of the blood, when allergics are exposed to "adequate" contact with the specific allergen. Widal's haemoclastic crisis, however, is not regarded as a specific allergic phenomenon, being observable in other, highly irritative states (24).

Leucopenia, already observed as an allergic phenomenon by v. Pirquet & Schick in 1905 (16) when studying the serum disease, was subsequently employed—specially by Vaughan (25)—as an aid to the diagnosis of certain foodstuff allergies; the reliability of Vaughan's "leucopenic index", however, is open to question.

Since the publication of v. Pirquet's and Widal's investigations, various workers when observing general allergic reactions, usually as individual observations, have found lowered blood pressure (2, 19), haemoconcentration (2, 13), leucopenia (5), acidosis (23) and, when there was severe oedema, reduction of

Blood examinations of 5 hay-fever

Sex Age No.	Time Min.	Hgb. % (Haldane)	Sedimentation rate mm/1. hr.	Coagulation time (Min.)	Thrombocytes c. mm.	Fibrinogen %
1.	0	95	3	3	372400	0.32
Male	15	85	—	—	—	—
16 years	30	95	3	1½	266000	—
4228/48	45	90	—	—	—	—
	60	95	3	3	358400	0.30
2.	0	98	4	2	224000	0.20
Male	15	94	—	—	—	—
47 years	30	92	4	2	248000	0.30
1852/47	45	92	—	—	—	—
	60	92	4	2¼	231000	0.33
3.	0	86	2	2½	302000	—
Female	15	87	—	—	—	—
16 years	30	86	6	2¾	255000	0.40
4516/46	45	84	—	—	—	—
	60	86	—	2½	317000	0.47
4.	0	102	2	3½	204000	0.21
Male	15	100	—	—	—	—
17 years	30	100	2	3	258000	0.27
1447/47	45	96	—	—	—	—
	60	97	2	3	283000	0.23
5.	0	90	2	3	195000	0.32
Male	15	87	—	—	—	—
19 years	30	87	2	3	158000	0.30
1363/47	45	87	—	—	—	—
	60	86	2	2½	182000	0.25

The first blood sample was taken 3 to 6 minutes after the onset of the shock (time 0), the others 15, 30, 45 and 60 minutes later. At 0, 30 and 60 mins. venous puncture for SR, cogulation time, thrombocyte count, fibrinogen determination, serum chlorine and serum-bicarbonate. The other tests were made on capillary blood.

serum chlorine (17). In some very few cases the blood coagulation time was found to be prolonged (21).

patients in acute allergic shock.

Serumchlor, m. equ./l.	Serum bicarb. m. equ./l.	Leucocytes c. mm.	Eosinophils c. mm.	Blood sugar mg. %	Pulse rate	Blood pressure mm Hg
104	22.2	8480	40	105	—	—
—	—	9880	0	105	—	—
102	20.0	6640	160	160	—	—
—	—	8840	40	—	—	—
99	19.9	6040	0	148	—	—
97	24.7	5080	40	115	130	130/95
—	—	11480	80	110	130	—
90	23.8	7680	0	130	106	125/90
—	—	6680	200	141	—	—
97	23.4	10760	0	139	96	125/90
94	26.2	4500	120	100	—	150/90
—	—	4100	80	95	—	—
93	25.9	5950	40	91	—	120/90
—	—	5450	80	88	—	—
97	26.5	5650	40	105	—	120/90
93	28.5	4840	0	124	100	100/—
—	—	4360	80	113	84	—
96	25.1	3280	40	99	—	120/—
—	—	—	40	96	—	—
99	25.9	4240	40	98	64	120/—
99	26.2	4560	80	111	76	120/85
—	—	4200	40	110	68	120/85
94	25.0	4260	40	84	76	130/90
—	—	6160	40	78	—	—
97	26.8	6080	80	91	—	130/90

In nearly every case the allergic shock conditions where these changes have been observed were caused by foreign serum; most frequently they were severe, protracted cases which in many instances terminated in death. To our knowledge, the only systematic investigations into acute allergic shock have been made by Waldbott, Ascher & Rosenzweig (26), who in 1939 examined the blood pressure, blood sugar and leucocyte number in a group

of hay-fever patients in whom a moderate overdose of pollen extract produced allergic shock—though never so violent as to call for ephedrine.

Sixteen patients had a general allergic reaction, and in the two hours tagen for the examination, the authors found leucopenia, lowered blood sugar, and hypotension.

OWN INVESTIGATIONS

For the purpose of making a closer study of the pathology of acute allergic shock, we have during the past three years for three months every spring, when the hay-fever patients come in for cutaneous tests before treatment, made preparations for examining the few patients who we know by experience may suffer from allergic shock on these occasions.

In all there are five patients whom we have succeeded in examining to such an extent that we consider the results will be of interest. In all cases the syndrome was the same: in varying periods after the allergen injection (8 to 25 minutes) there appeared conjunctivitis, nasal discharge, sneezing, general severe erythema with pruritus and, in the course of a few minutes, larger and smaller wheals all over the body, commencing on the neck. Four of the five patients acquired a dry cough five or ten minutes after the onset of the reaction, and it quickly developed into a rather wheezing dyspnoea; these four patients also had more or less pronounced facial oedema.

When the shock began, a tourniquet was immediately placed on the arm above the skin tests, and the first blood samples were taken from the other arm three to six minutes afterwards. Thereafter blood samples were taken every fifteen minutes. After just over an hour all the patients had fully recovered, except for a few wheals and the oedema, and were able to go home.

Our tests comprised the following:

Haemoglobin, sedimentation rate, coagulation time, serum chlorine, serum bicarbonate, thrombocytes, fibrinogen, leucocytes, eosinophil cells, blood sugar, as well as *pulse* and *blood pressure.*

The results will be seen from the table.

On account of their morbid condition patients Nos. 1 and 2 were given epinephrine between the first and second blood samples and showed a definite rise in the blood sugar values—the others showed a distinct fall followed by an incipient secondary rise. In these three latter patients there was also a definite, transient leucopenia with a fall of about 1000/c.mm.

All the other tests showed normal conditions with insignificant variations.

The point of particular interest is that haemoglobin, coagulability and thrombocyte count gave normal and unchanged values throughout the shock. The blood pressure unfortunately was not taken so frequently as was desirable, and not at all for Patient No. 1; a slight lowering at the onset of the shock was observed only in No. 4.

Accordingly, our patients were not shocked in the proper sense, nor was the prolonged coagulation time which is so characteristic of anaphylactic shock observed. On the other hand, the patients were very poorly on the whole; two had to have epinephrine, and it is well-known that the condition is dangerous and may lead to death in a short time, the patient being asphyxiated in the asthma paroxysm.

DISCUSSION

These subjects, then, are people with a strong, acute, general allergic reaction very reminiscent of the anaphylactic shock in the guinea-pig. In both cases the chief symptom is dyspnoea, but apart from leucopenia we have observed no agreement in the pathology of these conditions; the fall observed in the blood sugar, also described by Waldbott et al., is countered by a rise of the blood sugar in anaphylactic shock.

The cause of these differences lies presumably in the following:

The allergic shocks in our patients were all of short duration, and they subsided or were brought to an end before the condition had reached the danger line. We were thus unable to observe developments when a severe shock runs its full course.

It must also be pointed out that these allergic shocks were all of "the guinea-pig type", with dyspnoea as the outstanding symptom.

In our allergic consultation we have three times observed allergic shock after therapeutic overdosing of pollen extract in hay-fever patients, when the condition was very similar to anaphylactic shock in dogs:

Ten to twenty minutes after the allergen injection there were sudden alternating abdominal pains with diarrhoea and tenesmia; the patients turned pale and faint and sweated; in these patients the blood pressure fell to 60-70 mm.Hg. systolic. No other examinations were possible in these cases, and all three patients had to have epinephrine; this quickly improved their condition and at the same time the blood pressure rose to normal values.

Evidently these were allergic shocks with exactly the same etiology as those first described, but with another region of attack; the patients with the abdominal pains had no nasal discharge, sneezing, asthma paroxysms, or wheals. It may be that if blood tests could have been performed we might have found more pronounced changes.

CONCLUSION AND SUMMARY

In the course of moderate brief general allergic reactions in man, with dyspnoea as the outstanding symptom, leucopenia and a fall in the blood sugar over a short period are observed. A slight degree of lowered blood pressure may be found, but this is not the rule; there is no haemoconcentration, prolongation of coagulability or thrombopenia, and serum bicarbonate, serum chlorine, fibrinogen, sedimentation rate and eosinophil cells in the blood are all normal.

The danger of the examination prevented the observation of more severe and more protracted conditions of allergic shock. In general allergic reactions of the same etiology, but of the "dog type", there was a pronounced lowering of the blood pressure, and it is possible that in these cases—conforming with earlier in-

dividual examinations—there will be greater changes in the chemical and cytological composition of the blood.

Methods of examination.

Haemoglobin determination a.m. Sicca; *sedimentation rate* by Westergren's technique; *coagulation time* a.m. Howell-Gram; *thrombocyte count* a.m. Oluf Thomsen; *fibrinogen determination* a.m. Gram; *serum chlorine* after Johs. Clausen's modification of Volhard's principle; *serum bicarbonate* a.m. Van Slyke; *leucocyte count* after Ellermann's principle; *eosinophil cell count* a.m. v. Dungern; *blood sugar determination* a.m. Hagedorn-Jensen. *Blood pressure* measured with the Hg. manometer.

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From the Medical Out-Patients' Department of the Rigshospital, Allergy Consultation, Cogenhagen. (Director: Professor Eggert Moller, M.D.).

BIRCH POLLEN ALLERGY IN DENMARK

By

EGON BRUUN

During a visit to the allergy clinic in Stockholm in the spring of 1943, I saw the fairly numerous cases which were treated there for hay-fever due to birch pollen[1]. At that time birch pollen allergy had not been observed in Denmark, although there are indeed fairly many birch-trees in this country.

Dr. Juhlin-Dannfelt kindly placed at my disposal a quantity of Swedish birch pollen, and with it and a corresponding Danish preparation, all the hay-fever patients who applied to the allergy consultation of the Rigshospital were tested during the subsequent two years, but all the results were negative.

Shortly after the liberation in May 1945, a hay-fever patient applied for treatment to the allergy consultation; his hay-fever was caused by birch pollen. He presented strong, positive cutaneous reaction to birch pollen (but not to the usual kinds of grass pollen), and the exposure experiment with birch pollen gave a positive result.

In the course of the summer of 1945 and the spring of 1946, three other patients were examined and treated for birch hay-fever, and *all those four patients had lived as political refugees in Sweden during the years of 1943-45.*

These cases have not only the purely practical significance of demonstrating that birch pollen may give rise to hay-fever also in Denmark, but they are moreover of theoretical and scientific interest, because they are suggestive of the sensitization requiring a much greater quantity of allergen than does the inducement of

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From the Medical Out-Patients' Department of the Rigshospital, Allergy Consultation. Director: Professor Eggert Moller, M.D.

ALLERGIC EXAMINATION OF BAKERS AND MILLERS

BY

THOMAS CASTBERG and CHR. MYGIND SØRENSEN

INTRODUCTION

From ancient times it has generally been observed that attacks of dyspnoea and coryza occur among bakers and millers. Thus the mentioned frequent occurrence is discussed in *Ramazzini*'s book from 1700 about artisans' and artists' diseases. Even though some people thought that the attacks were due not only to mechanical causes, this conception was quite common up to the beginning of the twentieth century. It was indeed thought that flour-dust in connection with the secrete in the bronchial tree formed a cohesive mass which, by sticking to the mucous membrane particularly in the bronchi, gave rise to cough and dyspnoea. From the time about 1917 there are many works showing that flour can provoke bronchial asthma, vasomotor rhinitis, and Quincke's edema (*Talbot, Walker*). At that time the conception of asthma as an allergically conditioned disease began to assert itself, but for some years it was thought that it was not so much the very flour as its pollution which was the allergenic factor. Thus *Ancona* (1922) during an asthma "epidemic" among the workers of an Italian mill had found that they were sensitized to extract of flour, a closer examination revealing, however, that it was not the flour as such, but a mite which was found in the flour, to which they had been sensitized. *Storm van Leeuwen* also found such mites in Holland. However, *Grove* (1926) subsequently submitted *Ancona*'s cases to a closer examination and found that the flour gave rise to strong local irritation, whence she doubted the allergic etiology; those cases, in which pollution is the cause of the allergy, are generally

regarded as exceptions. In this connexion it must be mentioned that the number of cases of eczema in bakers rose considerably when he so-called flour-improving substances, such as potash bromate, ammonium persulphate etc. were added to the flour (*Haxthausen, Lomholt, Zitzke*). These substances are now very often regarded as the most frequent cause of eczema in bakers. In Denmark the use of ammonium persulphate is therefore prohibited by law on account of its eczematizing property.

The importance of exposure for the occurrence of flour allergy was stressed by *Berger* (1928) and *Schmidt* (1928). *Berger* found that more than half of the 37 millers and bakers composing his material were specifically sensitized to flour. *De Besche* in 1929 laid down asthma in bakers as a professional disease.

Baagoe (1933 and 1940) was the first who accounted for the frequency of flour-diseases amongst bakers. His material of 300 bakers shows that 33, *i.e.* 11 per cent of them suffered from vasomotor rhinitis or asthma. He mentions, however, that the discomfort as a rule was not severe.

Schwartz (1946) found that 7 out of 35 flour allergics who applied to the allergy clinic of the Rigshospital had been compelled to give up their work as bakers or millers on account of flour-coryza or flour-asthma. Thus it is reasonable to submit the incidence and gravity of flour-allergy to a closer examination, and we have endeavoured to elucidate this question by examining a number of able-bodied bakers.

Etiology, pathogenesis and symptomatology of flour-allergy previously having been described by *Baagoe* and quite recently by *Schwartz*, we shall abstain from a new description.

Flour-allergy manifests itself by symptoms from the respiratory passages (allergic rhinitis and bronchial asthma), from the skin (eczema, urticaria), from the subcutis (edemas), and finally symptoms from the central nervous system (migraine, epilepsy), as described by *Rowe*.

It is essentially a question of inhalation allergy (vasomotor rhinitis and asthma) and contact allergy (eczema). *Alimentary* flour-allergy is very rare in Denmark compared with U. S. A.

THE WRITERS' OWN MATERIAL

The aim of this work is to examine the frequency of flour-allergic diseases amongst *able-bodied* bakers.

On application to five bakeries employing 132 persons (122 bakers and 10 millers) altogether, we had the opportunity of obtaining complete *anamnestic data* from 130 persons, two refusing to give information; neither of these two according to their fellow-workers' reports had any symptoms of flour-allergy. Thirty-seven of the 130 individuals refused submitting to intradermal tests, but they were quite willing to give anamnestic information. Thus 95 bakers supplied us with anamnestic data and submitted to allergic examination. According to the anamneses of the 37 persons these form an even section of the total material, and as we have selected both the five bakeries and the test persons at random, the material must be regarded as being applicable.

The technique of examination.

The examinations consisted partly in the obtention of anamnestic data, partly in intradermal tests. The questions to be answered were as follows: (1) Have you any symptoms of allergic disease? and, in case of an affirmative reply, (2) What work and what sort of flour cause the greatest discomfort? (3) How strong are the symptoms? (4) Has any treatment been applied before? (5) Is there any familial disposition to allergic diseases?

For the intradermal tests were used extracts of the ordinary commodities such as rye-meal, wheat-meal, oat-meal and barley-meal in 0.1 ‰ solution. Moreover, intradermal tests were performed with extracts of mixed flour, *i.e.* equal portions of rye- and wheat-meal in 8 different concentrations (from $^1/_{10000}$ ‰ to 1 %), besides with extracts of yeast, leaven, mixed pollen, and two flour-improving substances, namely, monochloramine and potash bromate, i.e. 16 different tests altogether.

All the extracts were prepared by dispensing chemist Barfod (Frihavns Apoteket, Copenhagen), their sterility was controlled, and they were all found to be inactive on normal persons.

RESULTS

The anamnestic investigations:

Forty-two, *i.e.* 32.3 per cent of the 130 bakers reported having symptoms of allergic diseases when working with flour. The different allergic diseases are recorded in Table 1, which confirms the general conception that rhinitis, asthma and eczema are the most frequently occurring flour diseases. The high sensitizing percentages substantiate the assumption that the flour is the causal factor in the respective diseases.

TABLE 1.

Allergic diseases in 42 bakers.

The percentage figures represent the number of bakers in whom the respective disease was the *chief* complaint.

	Number of patients	Number of patients in whom the respective disease was the *chief* complaint	Percentage of the total material (130 bakers)
Vasomotor rhinitis	23	14	17.7
Asthma	14	13	10.7
Eczema	12	8	9.7
Urticaria	7	6	5,4
Hemicrania	2	1	
Intestinal allergy	1	0	
Total		42	

Intradermal reactions:

Ninety-three of the 130 bakers were willing to submit to the intradermal tests, and 39 of them (= 41.9 per cent) were found to suffer from skin allergy to flour and flour-improving substances. Among those 39 were 26 (= 67 per cent) who presented symptoms of allergic diseases, whereas 13 (= 33 per cent) had to be regarded as latent allergics.

Moreover, there were found 6 persons out of 65 (= 11.1 per cent), who presented symptoms of allergic diseases *without giving positive cutaneous reactions to the allergens applied.* In these cases it may be assumed that the allergic symptoms are due to

TABLE 2.

Intradermal reactions in 39 bakers with positive cutaneous reactions to flour and flour-improving substances.

	Number of patients	Number of patients with positive cutaneous reactions
Vasomotor rhinitis	21	21 = 100 %
Asthma	11	10 = 90.9 %
Eczema	8	7 = 87.5 %
Urticaria	4	3 = 75 %
Hemicrania	0	0
Intestinal allergy	1	1

hypersensitiveness to mites, house dust (not flour dust), moulds etc. Cutaneous reactions with these allergens were not, however, carried out.

What kinds of flour exert a particularly disposing action?

To elucidate this question the results of titration for each of the allergens applied are recorded in Table 3.

TABLE 3.

Quantitative and qualitative records of the positive reactions for each single allergen.

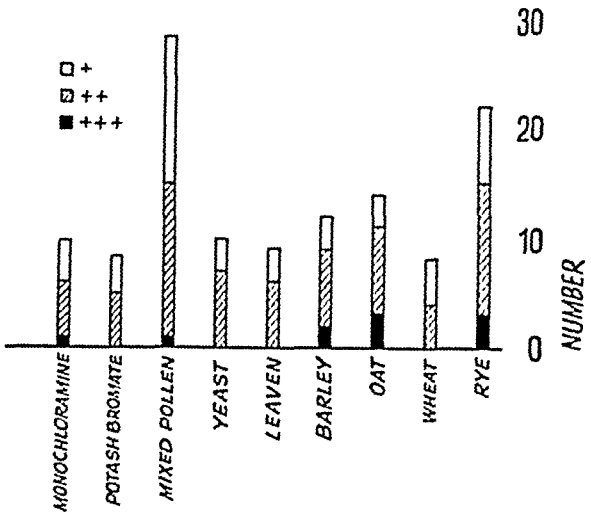


Table 3 shows—as was to be anticipated—that the majority of bakers are sensitized to rye-meal, which in Denmark is used the most. The same holds in Sweden, whereas the bakers in U.S.A. are mostly sensitized to wheat-meal, which is used the most there. Thus there does not seem to be any kind of cereal which acts particularly disposing. The exposure must be decisive. It is peculiar that mixed pollen is the allergen which gives the greatest number of positive reactions (see below).

Familial allergic disposition was found in 9 persons (6.9 per cent), seven of whom presented symptoms of flour allergy. We shall revert to this under the discussion.

DISCUSSION

As mentioned above, we found that 32.3 per cent of the 130 persons working with flour presented allergic symptoms. As is evident from Table 4, this figure is the highest percentage hitherto recorded.

TABLE 4.
Investigations in flour allergy.

	Number of persons tested	Symptoms of allergic diseases	Number of cutaneous reactions
Baagoe	300	33 = 11 %	54 % (scratch test)
v. Dishoek & Roux	262	66 = 25.2 %	66 = 25 % (scratch test)
Colmes et al.	32	9.4	15 = 47 % (intracut. test)
Hlavacek	70	7 = 10 %	7 = 10 % („ „)
Salén & Juhlin-Dannfelt	125	29 = 22.1 %	51 = 40.8 % („ „)
Castberg & Mygind	130	42 = 32.3 %	41.9 % (intracut. test)

All the materials of bakers and millers recorded in Table 4 were obtained in the same way as ours and should thus be comparable. It is seen that *Dishoek & Roux* find symptoms in 25 per cent of the cases, and *Salén & Juhlin-Dannfelt* in 23.2 per cent, which corresponds fairly well to the writers' 32.3 per cent. On the other hand, *Hlavacek, Baagoe,* and *Colmes et al.* only note

symptoms in about 10 per cent. It is worth mentioning, however, that *Baagoe* has been particularly interested in the symptoms from the respiratory passages; that does not explain the entire difference, however, for they are just those symptoms which dominate in the other materials. The difference is, perhaps, due to *Baagoe's* results having been obtained by letters. Hence he has not, perhaps, reached all the persons who have previously had symptoms, because they may have been symptomless at the moment of enquiry. *Hlavacek* seems merely to have been interested in the actually existing symptoms, and *Colmes and co-workers'* material is too small to permit of inferring anything definite from it. The result thus is that *from one third to one fourth of bakers and millers on account of allergic diseases experience constant or periodical discomfort at their work.*

The number of persons who showed positive intracutaneous reactions likewise appears from Table 4. *Salén & Juhlin-Dannfelt* and *Colmes et al.* record, more or less as do the writers, some 40 per cent with positive intracutaneous reactions, whereas *Dishock & Roux,* who used scratch tests, found, as was to be anticipated with that method, a lower figure (25 per cent), and on closer inspection of their results, another explanation of their low figure may also be possible, for 44 per cent of the bakers in small bakeries *(i.e.* bakeries employing less than ten persons) are sensitized, whereas only 12 per cent of 110 millers are sensitized. On the score of comparison it may be mentioned that our material includes ten millers, only one of whom is sensitized.

On the whole it may be said that *about two fifths of the bakers are sensitized to flour, whereas there probably are much fewer millers who are sensitized.*

Is it a question of an industrial disease?

It may here at once be stated that the persons who were examined did not present any disabling industrial disease, our material only showing cases with slighter discomforts, as had to be anticipated, because it consisted of *able-bodied* bakers and millers, and the severely affected persons had changed their trade. *Schwartz,* who has published the reports of the Invalidity Insurance Council from 1928 to 1942 (2015 cases), among the invalids

found 7 bakers, 1 confectioner and 3 millers suffering from disabling bronchial asthma with relation to their work, but from the Invalidity Insurance Council's reports it does not appear whether it was a question of flour allergy, because only three patients had been submitted to cutaneous reactions (scratch tests), and in one case only the reaction had been positive.

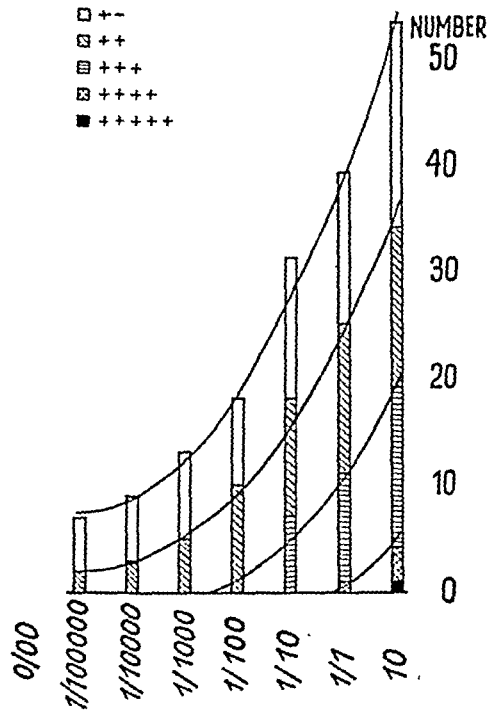
Now it is of interest to see how many of those individuals, in whom it was impossible objectively to demonstrate specific sensitization, experienced subjective symptoms. Such symptoms were indeed found in 17 of the 54 persons who reacted negatively. Closer examination of these cases revealed, however, that seven of them reacted positively to pollen or leaven, and for the rest it holds that their symptoms were without sure relation to the work.

Dishoek & *Roux* report that only "some few" of the non-sensitized individuals had symptoms, and *Salén* & *Juhlin-Dannfelt* found that five (6.8 per cent) out of 74 persons with negative intracutaneous reactions had symptoms. It is thus a question of small numbers, so small that anyhow they cannot shake the assumption of flour or flour-improving substances being of importance for the allergic diseases with which we have dealt, so much the less as but few antigens have been applied. If a thorough allergic diagnosis including all possibilities could have been carried through (the examinations were performed on the working premises), the apparently puzzling cases might, perhaps, have been explained too.

As was mentioned, the greatest sensitization was found for rye, which is used the most in Denmark. *Salén* & *Juhlin-Dannfelt* in accordance with this also found the greatest sensitization for rye in Sweden, whereas American authors (*Rowe*, and *Eyerman*) report the greatest sensitization for wheat, which, in U.S.A., plays the same part in the diet as does rye in Denmark. *Schwartz*' patients, who are Danish, are sensitized in an almost equal degree to rye, oats and wheat, but they were chiefly persons who, being very poorly, after long-standing disease applied to the policlinic, whereas our examinations were performed on able-bodied workers. It may therefore be presumed that the allergy of *Schwartz*' patients has been more polyvalent.

In connection with the qualitative examination of the sensitization (Table 3) it was also endeavoured to determine it quatitatively. The result is illustrated by Curve 1.

Curve 1.
Quantitative examination of the sensitization.



On the ordinate axis is noted the number of persons who react positively to intracutaneous injection of extract of mixed flour, and on the abscis axis, the corresponding concentrations of the extracts applied. All the plotted curves are parts of parables, and it is evident that they ascend strongly with increasing concentrations. This ascent is suggestive of the sensitization among the tested persons being exceedingly common opposite the higher concentrations, but that, in the great majority of the workers, it does not reach such degrees as will give rise to discomfort. It must be emphasized that non-allergists do *not* react positively to the respective extracts. The trend of the curves quite agrees with the work-

ers being employed in an industry which may entail specific sensitization. The fact that the curves ascend more abruptly than corresponding to a logarithmic ascent, in our opinion is suggestive of the normal resistance against sensitization among the workers examined.

From Table 3 it is evident that the great majority of the workers (31) show positive reactions to mixed pollen extract. This is scarcely attributable to pollution of the substances used for the preparation of the extracts, which were examined and found to be microscopically pure, but sooner to common antigens in pollen and cereals. A similar finding was reported by *Dishoek & Roux,* who, among 66 persons with positive intracutaneous reactions, found 27 who reacted positively also to pollen. *Salén & Juhlin-Dannfelt* found 6 among 65, *i.e.* much fewer, whereas *Schwartz* found 3 among 35. However, only one of the persons examined here presented strong, positive reaction to pollen, and as hay fever patients always react very strongly positively to pollen extract, the numerous positive reactions to pollen in flour-allergists probably are due to but little dominant common antigens.

Nine persons reacted to extracts of leaven, and eight of these also reacted to extracts of yeast, whilst none reacted to yeast alone. None according to what was reported had been in contact with yeast. One single person had a distinct positive reaction to leaven as only positive reaction, and he had no discomfort. All the remaining eight persons who reacted positively to both leaven and yeast, presented distinct positive reactions (++ or more) to at least two of the other extracts, and half of them even reacted to all the extracts, the tested persons thus representing a material of polyvalent sensitization. Two of the men many years ago had had transitory spells of eczema (one of them reacted to monochloramine). All the others had vasomotor rhinitis, asthma, or both, and, as was mentioned, they reacted to several other substances. Even though nothing definite can be inferred from this complicated picture, the circumstance that only one of the men was sensitized to leaven alone, indicates distinctly that leaven plays a very inferior rôle in the case of allergic diseases in bakers.

Sensitization to the flour-improving substances monochloramine and potash bromate, which are used in Denmark, has also been examined (Table 3). Five persons were sensitized to monochloramine, and two of these had eczema, whereas one had suffered from it 20 years ago; four men had rhinitis, whereas one had no symptoms at all. With this one exception they were all sensitized to flour. Something similar holds for those 8 men who were sensitized to potash bromate, for two had eczema, one of them had no symptoms, and the rest had asthma or rhinitis. All were at the same time sensitized to flour. The part of the flour-improving substances as cause of eczema has been established by numerous authors (*Haxthausen, Lomholt, Zitzke, Bonnevie, Salén*), nor does our little material contradict the fact. However, in proportion to flour the improving substances play a very inferior part as causal agents of the symptoms, as has also been emphasized previously by *Dishoek & Roux*. That does not hold for ammonium persulphate, however, eczema mostly being due to this substance, and that is why its use as flour-improving substance is forbidden in several countries, e.g. in Denmark.

Among *Baagoe*'s 45 patients with flour allergy were found 11 (24.4 per cent) whose nearest relations (parents, brothers and sisters) suffered from allergic diseases, flour allergy being found in 4 (= 8 per cent) of them. *Hlavacek* mentions that, if persons incur symptoms, they probably have an allergic disposition, and that such persons have but little power of resistance to sensitization, if they are exposed to a strong antigen influence. *Schwartz* found that 52 per cent of the allergics had a familiar allergic disposition (parents, grand-parents, brothers and sisters or paternal and maternal uncles and aunts). The reason why we do not find any corresponding figures, may, perhaps, be due to our not having focussed sufficient attention on this possibility, for empirically many persons do not recollect diseases in their families till they have had time to reflect more thoroughly.

The conclusion thus is that also *able-bodied* bakers, on account of the massive exposure to the flour allergen, frequently are sensitized to flour and incur some slight discomfort at their work with flour.

SUMMARY

130 bakers and millers employed in five different bakeries in Copenhagen selected at random were submitted to examination in order to establish the incidence of flour-allergic diseases among able-bodied bakers.

Among 130 bakers were found 42 who had symptoms of allergic diseases during their work with flour. In 14 of them asthma was the chief complaint, whereas in 13 others it was vasomotor rhinitis, in 8, eczema, 6 urticaria, and in one of them it was hemicrania.

Ninety-three of the 130 bakers were willing to submit to intradermal tests, and the result was that 39 (= 41.9 per cent) were found to be skin-allergic to flour or flour-improving substances. Twenty-six (= 66.6 per cent or 20 per cent of the total number of 93 persons submitted to intradermal tests) of these 39 persons presented symptoms of allergic diseases, whereas 13 (= 33.3 per cent or 13.9 per cent of the total number of 93 persons submitted to intradermal tests) must be regarded as latent allergics.

Five out of 54 bakers with negative reactions presented symptoms of allergic diseases, it must be emphasized, however, that the patients were only examined with certain allergens (flour, flour-improving substances, and pollen). Hence the symptoms of those five negatively reacting individuals must be assumed to be due to other allergens, e.g. to dust.

In Denmark rye apparently is particularly apt to give rise to allergic diseases.

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DISCUSSION: K. BAAGØE, P. BONNEVIE

Baagoe thanks for the intersting lecture. Might not the circumstance that there are so many patients with eczema who present positive cutaneous reactions to flour, be due to their suffering from baker's coryza or asthma?

A material of patients as yours—just as that which I have published previously, does not *per se* show how severe an affection bakers' asthma is, for the bakers who have been compelled to give up their work, are not included in the material.

Bonnevie, P.: The urticarial flour allergy demonstrated in cases of eczema is scarcely the primary cause of the skin affection, but is due to a simultaneous sensitization of the respiratory passages, manifest or latent. An eczema due to other causes must, however, be assumed to exacerbate and persevere by contact with flour, when the flour in case of existing allergy can induce cutaneous reactions in the fissured and eroded skin—just as in scratch tests.

The combination of bakers' eczema and bakers' asthma has contributed to the Danish Workmen Compensation Act—probably

the first—among the compensated occupational diseases including a number of cases of asthma (flour, fur colours, synthetic resins, exotic kinds of wood), as it was considered unreasonable only to compensate the skin affection.

The end of the Transactions of the First Northern Congress of Allergy.

Acta allergologica, 1948, I, 297—310.

Travail de la Clinique Dermatologique Universitaire de Genève
(Dir. Prof. Dr. W. Jadassohn)

SUR DES ANTIGENES DU PENICILLIUM NOTATUM

Par

H. ISLER et A. KARABADJAKIAN

En 1924, Bloch, Labouchère et Schaaf ont isolé de cultures d'Achorion quinckeanum un produit qu'ils ont nommé trichophytine sèche („Trockentrichophytin") contenant les substances insolubles dans l'alcool méthylique et solubles dans l'eau. D'après ces auteurs, ce produit est constitué en majeure partie de polysaccharides azotés. Il s'agit de la fraction „C", comme pour les produits ultérieurement isolés de cultures de différents germes (Avery et collaborateurs (1925), Tomesik et Kuretschkin (1928), Lancefield (1928), Kesten et Mott (1931), Enders (1929), Laidlaw et Dudley (1925)).

W. Jadassohn, Schaaf et Sulzberger ont fait sur la trichophytine sèche des expériences selon la méthode de Schultz-Dale et ont pu démontrer que:

1. L'utérus isolé d'un cobaye infecté par l'Achorion quinckeanum réagit spécifiquement in vitro à la trichophytine sèche isolée d'une culture de ce champignon.

2. Lorsqu'on injecte à un cobaye de la trichophytine sèche, après un certain temps d'incubation, l'utérus isolé de l'animal réagit spécifiquement in vitro à ce produit. La trichophytine sèche contient donc un ou plusieurs antigènes complets.

W. Jadassohn, Schaaf et leurs collaborateurs ont montré ultérieurement par un grand nombre d'expériences que la trichophytine sèche est un mélange de différents antigènes. Par exemple, lorsqu'on sensibilise un cobaye en lui injectant de la trichophytine sèche isolée d'une culture d'Achorion quinckeanum, l'utérus isolé

de l'animal réagit spécifiquement in vitro à une trichophytine sèche isolée d'une culture de Trichophyton gypseum. Cette réaction peut être neutralisée, en ce sens que l'utérus mis en présence de la substance ne se contracte que la ou les premières fois, puis ne réagit plus. Si on le met alors en présence de la trichophytine sèche ayant servi à le sensibiliser, il se contracte de nouveau et cette contraction peut, à son tour, être neutralisée.

Les auteurs ont fait des expériences semblables avec différents champignons et avec d'autres produits. L'explication qu'ils donnent de leurs résultats est que les trichophytines sèches sont des mélanges d'antigènes. Ainsi dans l'exemple cité plus haut, le produit isolé de la culture d'Achorion quinckeanum doit contenir au moins deux antigènes. L'un d'entre eux se trouve également dans le produit isolé de cultures de Trichophyton gypseum, tandis que l'autre est propre à l'Achorion quinckeanum et ne se rencontre pas chez le Trichophyton gypseum.

Les auteurs ont encore montré que différents champignons appartenant à la famille des hyphomycètes produisent, à côté des antigènes qui leur sont propres, un antigène commun. Toutefois il n'ont pu assurer, jusqu'à maintenant, que cet antigène commun est produit par tous les hyphomycètes et qu'il n'est produit que par eux. Ils ont montré qu'une oïdiomycétine sèche provoquait parfois une réaction chez les animaux sensibilisés avec la trichophytine, mais qu'en revanche, la trichophytine sèche ne donne chez les animaux sensibilisés avec l'oïdiomycétine sèche que des réactions douteuses.

Il nous a semblé intéressant à plusieurs points de vue d'isoler de cultures de Penicillium notatum la substance correspondant à la trichophytine sèche. Nous appelerons cette substance „microbine du Penicillium notatum".

PREPARATION DE LA MICROBINE SECHE DU PENICILLIUM NOTATUM*

Nous nous sommes référés, pour préparer la microbine sèche

* Souche Westling, aimablement mise à notre disposition par l'institut de Botanique de Genève (prof. Chodat).

du Penicillium notatum, à la méthode de préparation de la trichophytine sèche de Bloch, Labouchère et Schaaf.

Nous avons modifié cette méthode en ce sens que le champignon a été cultivé sur un milieu ne contenant que des substances de constitution chimique connue, dans le but d'éliminer tout apport d'antigène étranger. Nous avons utilisé le milieu de Cook et Brown dont la constitution est la suivante:

$NaNO_3$	0,3 g
KH_2PO_4	0,05 g
$MgSO_4 \cdot 7\ H_2O$	0,025 g
NaCl	1,0 g
Lactose	3,0 g
Eau	100,0 g

Le Penicillium notatum y pousse très bien. Il croît en surface et devient blanc et duveteux tandis que le liquide prend une coloration jaune or. Nous l'avons laissé se développer pendant 62 jours à 20°. Le liquide du milieu de culture a ensuite été décanté et filtré. La masse solide a été broyée par agitation mécanique dans une faible quantité de liquide, et la pâte ainsi obtenue filtrée. Les deux filtrats réunis ont été concentrés au vide (15 mm) et à 50—60°, jusqu'à l'obtention d'un volume égal au 1 à 2 % du volume initial. Le résidu a été repris à plusieurs reprises par de petites quantités d'eau, jusqu'à obtention d'un volume égal aux 5 % de la quantité initiale. Cette solution filtrée a été versée goutte à goutte dans 8 fois son volume en alcool méthylique absolu, en agitant. Un précipité beige clair s'est formé. Après un repos de 24 heures la solution a été filtrée et le précipité lavé 5 fois au méthanol, essoré, et séché au vide, à froid, jusqu'à poids constant. Le produit a été utilisé tel quel pour nos expériences, sans autre purification préalable.

PREPARATION DE LA TRICHOPHYTINE SECHE DE L'ACHORION QUINCKEANUM

Nous avons eu besoin pour nos expériences de la trichophytine sèche de l'Achorion quinckeanum. Nous l'avons préparée de la

même façon que celle de la microbine du Penicillium notatum, sauf en ce qui concerne le milieu du culture. Nous n'avons en effet pas pu utiliser un même milieu de culture pour les deux champignons car l'Achorion quinckeanum se développe difficilement sur le milieu que nous avons utilisé pour le Penicillium notatum et ce dernier ne pousse que très lentement sur le milieu que nous avons choisi pour l'Achorion quinckeanum, milieu de Rippel et Lehmann, modifié par W. Jadassohn, Fierz et Huber, dont la composition est la suivante :

Glycocolle	0,1 %
Glucose	1,0 %
$MgSO_4$	0,01 %
K_2HPO_4	0,3 %
$FeSO_4$	0,01 %
$MnSO_4$	traces
$CuSO_4$	traces

L'Achorion quinckeanum s'est également développé pendant 62 jours à 20° et la culture a été traitée de façon identique à celle décrite pour le Penicillium notatum. Le produit obtenu à la même apparence que la microbine du Penicillium notatum. Il n'a pas non plus été purifié davantage.

PROPRIETES ET REACTIONS

Les deux préparations sont solubles dans l'eau, la trichophytine plus facilement que la microbine du Penicillium notatum. Les solutions sont colorées en jaune. Les diverses réactions effectuées sur les deux produits figurent dans le tableau I.

Comme le montre le tableau I, les deux produits ont des réactions semblables. Ils contiennent des hydrates de carbone (réaction de Molish positive) qui ne sont pas le glucose (respectivement le lactose) des milieux de culture (réaction de Fehling et de Bénédict négatives). Nous n'avons pas pu mettre en évidence la présence de protéines (réaction du Biuret négative). Comme la trichophytine sèche, la microbine du Penicillium notatum semble être un polysaccharide azoté. Nous devons faire remarquer

que la réaction à l'iode est négative alors qu'elle était positive pour le produit préparé par Bloch, Labouchère et Schaaf. Les deux produits ne présentent aucune activité antistaphylococcique (test sur plaque d'agar).

TABLEAU I

Reaction	M.P.N.	T.
Biuret	—	—
Acide sulfosalicylique	—	—
Millon	—	—
Xanthoprotéique	+	+
Molish	+	+
Fehling	—	—
Benedict	—	—
Iode	—	—
Recherche de N	+	+
„ „ S	—	—
Activité antistaphyloccocique	—	—
PH (solution aqueuse à 5 %)	8	5,5

M.P.N. = microbine du Penicillium notatum.
T. = trichophytine sèche de l'Achorion quinckeanum.

EXPERIENCES DE SCHULTZ-DALE

Pour résoudre différentes questions se posant au sujet de la microbine du Penicillium notatum, nous avons eu recours à la méthode de Schultz-Dale, consistant à injecter au cobaye la substance à étudier, à isoler après un certain temps d'attente les cornes de l'utérus et à étudier le comportement de celles-ci en présence de la substance injectée.

Propriété antigénique de la microbine du Penicillium notatum.

La première question que nous nous sommes posée était de savoir si la microbine du Penicillium notatum est un antigène, c'est à dire si elle est capable de sensibiliser le cobaye de façon

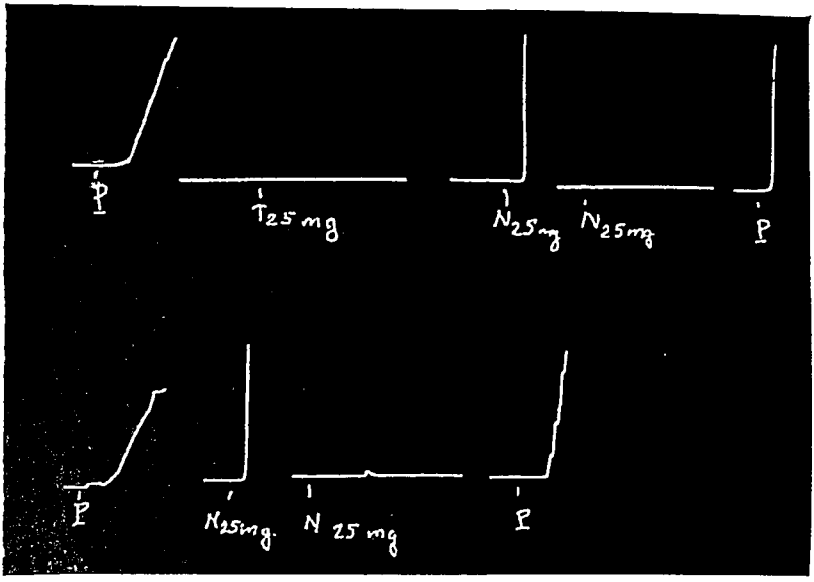

Figure 1

Réaction de Schultz-Dale sur les deux cornes d'utérus du cobaye No 489, sensibilisé par 25 mg de microbine du Penicillium notatum.

P = pituglandol.

T = trichophytine sèche.

N = microbine du Penicillium notatum.

à ce qu'il réagisse à cette même substance, comme c'est le cas pour la trichophytine sèche. Pour répondre à cette question, nous avons injecté à 6 cobayes, par voie sous-cutanée, 25 mg de cette substance. Après un temps d'incubation variant entre 33 et 37 jours, les utérus ont été prélevés et examinés dans l'appareil de Schultz-Dale. Chez quatre cobayes, les utérus mis en contact avec la microbine du Penicillium notatum (25 mg dans 50 cc de solution de Tyrode) se sont contractés, et cette contraction a pu être neutralisée (fig. 1 et 2). Chez 2 cobayes la réaction a été négative, de même que chez les cobayes non sensibilisés.

Nous pouvons donc conclure de cette expérience que la substance isolée de la culture du Penicillium notatum est capable de sensibiliser le cobaye et de déclencher une réaction spécifique, comme les trichophytines sèches.

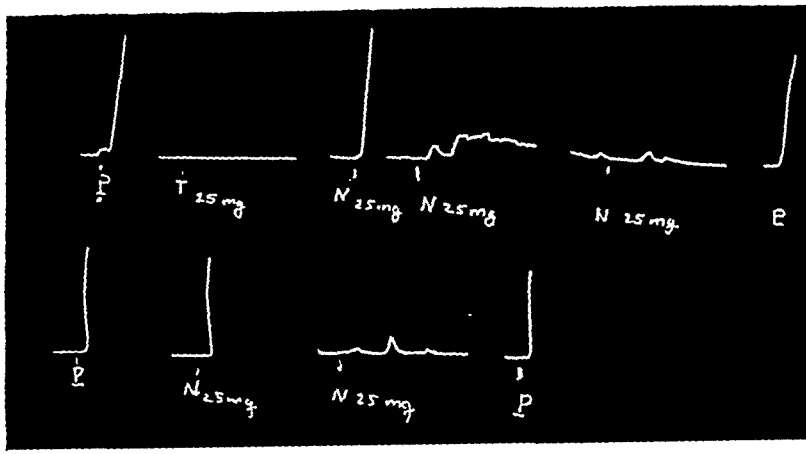

Figure 2

Réaction de Schultz-Dale sur les deux cornes d'utérus du cobaye No 488, sensibilisé par 25 mg de microbine du Penicillium notatum.

P = pituglandol.

T = trichophytine sèche.

N = microbine du Penicillium notatum.

Relations antigéniques entre la microbine du Penicillium notatum et la trichophytine sèche de l'Achorion quinckeanum.

La deuxième question qui se posait était de savoir si la microbine du Penicillium notatum et la trichophytine sèche de l'Achorion quinckeanum possèdent un antigène commun.

L'intérêt théorique de la présence éventuelle d'un antigène commun à deux champignons avait déjà suscité des recherches qui avaient montré, comme nous l'avons mentionné, que différents hyphomycètes produisent un antigène commun ; par contre, les expériences faites en vue de mettre en évidence une communauté d'antigène entre les hyphomycètes et les oïdiomycètes n'avaient pas été très concluantes.

Mais à côté de l'intérêt purement théorique, la question en présente un d'ordre pratique. En effet on parle actuellement beaucoup de réactions secondaires à la pénicilline et l'on a observé, en particulier, que ces réactions se rencontrent dans un pourcentage beaucoup plus élevé chez les personnes ayant eu une dermatomycose que chez les autres. Par exemple les observations de

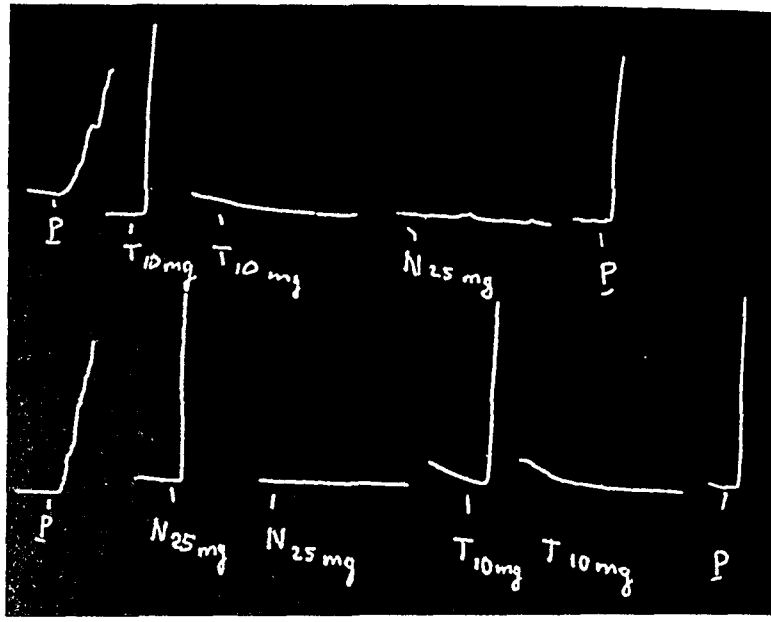

Figure 3

Réaction de Schultz-Dale sur les deux cornes d'utérus du cobaye No 426, sensibilisé par 25 mg de trichophytine sèche.

P = pituglandol.

T = trichophytine sèche.

N = microbine du Penicillium notatum.

Graves, Carpenter et Unangst (1944), de Binkley et Brockmole (1944), de Cormia et collaborateurs (1945, 1946, 1947), de Fromer (1947), de Peck et Siegal (1947) et de Farrington et Tamura (1948) tendent à montrer qu'il existe une relation étroite entre l'hypersensibilité à la pénicilline et les dermatomycoses. Il nous a semblé intéressant de rechercher s'il existait quelque chose de commun entre les champignons provoquant les dermatomycoses et le Penicillium notatum. Dans ce but, nous avons injecté à 6 cobayes, par voie sous-cutanée, 25 mg de trichophytine sèche. Après un temps d'incubation variant entre 33 et 36 jours, les utérus des 5 cobayes survivants ont été prélevés et examinés dans l'appareil de Schultz-Dale. Les animaux se sont montrés sensibilisés à la trichophytine sèche (fig. 3 et 4).

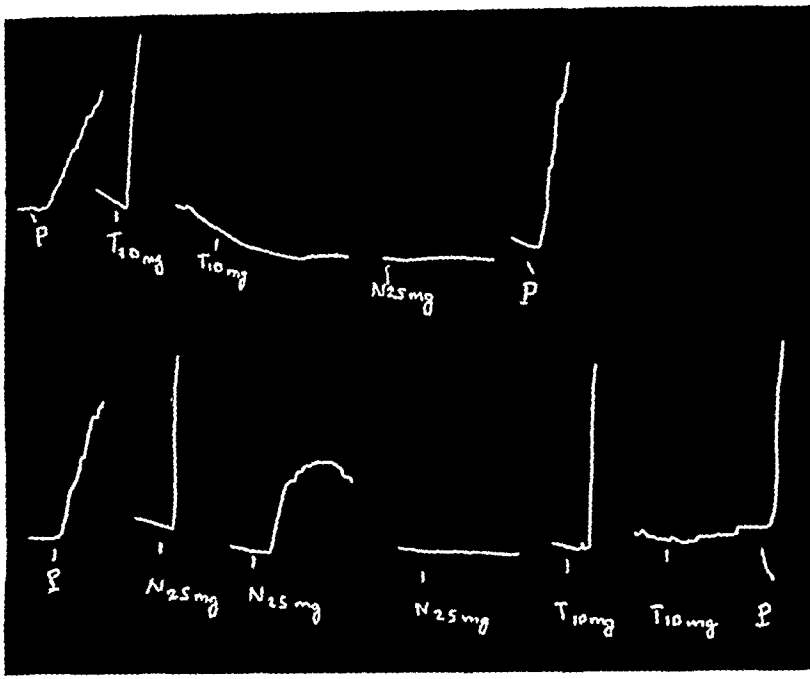

Figure 4

Réaction de Schultz-Dale sur les deux cornes d'utérus du cobaye No 425, sensibilisé par 25 mg de trichophytine sèche.

P = pituglandol.

T = trichophytine sèche.

N = microbine du Penicillium notatum.

Les utérus de 4 de ces animaux ont été mis en présence de la microbine du Penicillium notatum et 3 ont montré une réaction spécifique et neutralisable à ce produit (figures 3 et 4).

La trichophytine sèche a donc sensibilisé des cobayes à la microbine du Penicillium notatum. *Ces deux substances doivent contenir, par conséquent, un antigène commun.*

L'utérus, après contraction à la microbine du Penicillium notatum et neutralisation a encore donné une réaction spécifique et neutralisable à la trichophytine sèche (figures 3 et 4). La trichophytine contient donc, en plus de l'antigène commun, un antigène propre qui ne se trouve pas dans la microbine du Penicillium notatum.

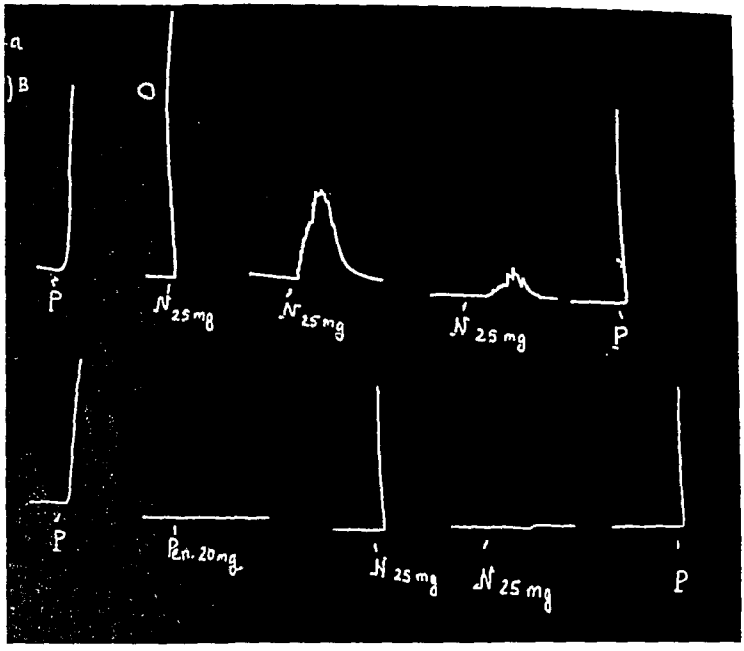

Figure 5

Réaction de Schutz-Dale sur les deux cornes d'utérus du cobaye No 234, sensibilisé par 25 mg de microbine du Penicillium notatum.

P = pituglandol.

Pen = Penicilline G cristallisée.

N = microbine du Penicillium notatum.

Ces résultats sont en accord avec les travaux antérieurs faits sur divers champignons.

Nous avons également fait l'expérience inverse, consistant à mettre en présence de la trichophytine sèche l'utérus du cobaye sensibilisé à la microbine du Penicillium notatum. Des 4 cobayes réagissant à cette dernière substance, aucun n'a donné de réaction à la trichophytine sèche (fig. 3 et 4).

On peut expliquer les deux résultats apparemment en contradiction de deux manières :

a) Les deux substances contiennent un antigène commun qui, dans la microbine du Penicillium notatum, se trouve en quantité suffisante pour déclencher la contraction de l'utérus mais en quantité trop faible pour sensibiliser le cobaye.

b) Les deux substances contiennent un antigène commun, présent dans la trichophytine sèche à l'état d'antigène complet, et dans la microbine du Penicillium notatum à l'état d'antigène incomplet, capable de déclencher mais incapable de sensibiliser (haptène).

Il nous a paru intéressant de déterminer encore si les utérus des cobayes sensibilisés par la microbine du Penicillium notatum réagissaient à la pénicilline G cristallisée. Nous avons fait l'expérience sur deux cobayes et n'avons pas obtenu de contraction (20 mg de pénicilline). Après la pénicilline, les utérus ont donné une contraction à la microbine du Pénicillium notatum (fig. 5).

La pénicilline a donné le même résultat négatif chez 2 cobayes sensibilisés par la trichophytine sèche.

La streptomycine (100 mg) a également donné un résultat négatif chez les cobayes sensibilisés par la microbine du Penicillium notatum et par la trichophytine sèche.

COMMENTAIRES

En résumé nos expériences ont montré que le Penicillium notatum produit une substance soluble dans l'eau, insoluble dans l'alcool méthylique, et capable de sensibiliser le cobaye de façon telle qu'on obtient une réaction de Schultz-Dale positive. Cette substance n'a pas sensibilisé le cobaye à la trichophytine sèche. Par contre la trichophytine sèche a sensibilisé le cobaye à la microbine du Penicillium notatum. Ces deux champignons produisent donc un antigène commun.

Cela explique de façon très satisfaisante l'hypersensibilité d'individus atteints de dermatomycoses, à des substances produites par le Penicillium notatum, mais n'explique pas une hypersensibilité à la pénicilline pure (100 %). On a récemment prétendu que la pénicilline pure est un faible antigène (Peck et Siegal) et que « Anaphylactic sensitization to crystalline sodium penicillin G may be induced in the guinea pig by infection of the skin with T. gypseum ». (Cormia, Lewis et Hopper). Peck par contre écrit : « We are unable to prove anaphylaxis either to trichophy-

tin or penicillin in the guinea pig in the course of T. gypseum skin infection », puis plus loin : « ... is further evidence of the fact that penicillin and trichophytin do not have a common antigen ».

Nous ne pouvons pas nous prononcer sur ces résultats, mais nous tenons à souligner que l'antigène que nous avons isolé de cultures de Penicillium notatum n'est pas de la pénicilline. (Il ne présente aucune activité antistaphyloccocique ; de plus il est obtenu par précipitation dans l'alcool méthylique, dans lequel la pénicilline est soluble). Il est possible, d'après nos expériences, que les dermatomycoses puissent sensibiliser des individus à des impuretés contenues dans la pénicilline. Il n'est peut-être pas exclu que même la pénicilline cristallisée contienne en traces des impuretés communes au champignon pathogène et au Penicillium notatum.

RÉSUMÉ

1. De cultures de Penicillium notatum sur milieu synthétique, on peut isoler un produit contenant les substances solubles dans l'eau et insolubles dans l'alcool méthylique. Ce produit correspond à la trichophytine sèche de Bloch, Labouchère et Schaaf (« Trockentrichophytin »), et contient probablement un polysaccharide azoté. Il ne présente aucune activité antistaphylococcique.
2. Ce produit est capable de sensibiliser le cobaye.
3. Les cobayes sensibilisés avec ce produit n'ont pas réagi à la trichophytine sèche isolée d'une culture d'Achorion quinckeanum sur milieu synthétique.
4. Les cobayes sensibilisés par la trichophytine sèche de l'Achorion quinckeanum ont réagi au produit isolé de la culture du Penicillium notatum. Le Penicillium notatum et l'Achorion quinckeanum produisent donc un ou plusieurs antigènes communs.
5. Les cobayes sensibilisés par le produit isolé de la culture de Penicillium notatum ou par la trichophytine sèche de l'Achorion quinckeanum n'ont réagi ni à la pénicilline G cristallisée ni à la streptomycine.

SUMMARY

(1) From cultures of Penicillium notatum on synthetic medium can be isolated a product containing the substances soluble in water but insoluble in methyl alcohol. The product corresponds to Bloch, Labouchère and Schaaf's dry trichophytin ("Trockentrichophytin"), and probably contains nitrogenous polysaccharide. It does not present any antistaphylococcic activity.

(2) This product is able to sensitize the guinea-pig.

(3) Guinea-pigs sensitized with this product do not react to dry trichophytin isolated from a culture of Achorion quinckeanum on a synthetic medium.

(4) Guinea-pigs sensitized with dry trichophytin of Achorion quinckeanum have reacted to the product isolated from Penicillium notatum. Penicillium notatum and Achorion quinckeanum thus produce one or several common antigens.

(5) Guinea-pigs sensitized by the product isolated from the Penicillium notatum culture or by the dry trichophytin of Achorion quinckeanum have not reacted either to crystallin penicillin G or to streptomycin.

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From the University Clinic of Dermatology, Copenhagen
(Chief: Professor H. Haxthausen, M.D.).

VITALITY OF EPIDERMAL CELLS AFTER ALLERGIC EXPOSURE

Studied by Means of a Special Transplantation Technique

By

H. HAXTHAUSEN

INTRODUCTION

In a preceding work[1] I have tried by means of skin transplantation on uniovular twins to elucidate the nature of the hypersensitiveness in allergic eczema. The principle of those experiments was to sensitize one of a pair of twins (A) with dinitrochlorbenzene and after development of the hypersensitiveness to transplant a piece of skin from A to the non-sensitized twin (B), and vice versa. After healing of the transplants, patch tests were performed, with uniform outcome in experiments on two pairs of twins: after healing on the non-sensitized twin (B), the skin from the sensitized twin (A) loses its capacity for reaction, while, conversely, skin from the untreated twin (B) acquires hypersensitiveness when it has healed on A. The experiments indicate that in allergic eczema th hypersensitivity is not limited to the epidermal cells themselves but depends on a factor, more likely an antibody, with which these cells are supplied by way of the blood stream. This assumption was confirmed through parabiotic experiments on guinea-pigs,[2] in which it proved possible to transmit the hypersensitiveness from an already sensitized animal to its untreated partner.

These transplantation experiments were carried out on uniovular twins because homotransplantation of skin—*i.e.* from one person to another—generally does not result in permanent healing, even if the two individuals be closely related. Evidently the speci-

[1] Acta derm. 23: 438, 1943.

[2] ibid. 24: 286, 1944.

ficity of the skin is very highly developed, and only when the relationship is so close as that of uniovular twins does the transplantation last just as certainly as in autotransplantation. For obvious reasons, however, it would be rather difficult to carry out such twin transplantations on a fairly large scale.

In some transplantation experiments carried out for entirely different purposes, however, a number of observations showed that, under particular conditions, epidermal homotransplantation is not only possible, but may even be carried through successfully in most of the cases, affording thus a more abundant chance to study the nature of eczematous-allergic hypersensitiveness.

When homotransplantation of skin is said above generally to turn out a failure, this has reference to the grafts usually employed where attempts are made to make large patches of skin heal directly on granulation tissue.

In the transplantation experiments that will be mentioned in the following, tiny transplants of the pinch graft type were employed, and these experiments were originally performed in order to see whether different combinations of blood types in donor and receptor might influence characteristically the behavior of homotransplanted grafts. The result was negative in so far as no difference was found regularly in relation to the blood types. At the same time, however, it turned out that with the technique employed it was possible in most of the cases to obtain a quite uniform growth of homologous and autologous grafts, at any rate for a couple of weeks, in many cases even an apparently lasting take of the homologous graft. Accordingly, it should be possible in this way to investigate, for instance, how skin with eczematous-allergic hypersensitiveness would behave after transplantation on a non-hypersensitive individual—besides many other questions of interest to the pathogenesis of allergic eczemas.

TECHNIQUE

As a rule the soil for the transplantations has consisted in large varicose ulcers or burns in patients confined to bed. In the following, these patients will be designated as *receptors*.

By bandaging with ¼ % chloramine compress for some length of time, an attempt was made to produce a fresh red granulation surface. The grafts were taken from the receptor himself and from patients with allergic eczema; and in the following the latter are designated as *donors*. Prior to the transplantation in every instance, the donors have been examined clinically and serologically for syphilis and other diseases that possibly might involve a risk of transmission.

A modification of the so-called pinch graft technique has been employed, with small pieces of skin placed side by side on the granulation surface. The grafts were made as small as possible which is accomplished most easily by lifting a small part of the skin by means of a sharp curved needle and then, with a pair of small curved scissors, cutting off the graft just below the point of the needle. This gives small elongated grafts of about 1 × 2 mm., resting on the tip of the needle.

Histological examination has shown that these tiny pieces of skin, besides epidermis, contain merely the most superficial part of the corium. The grafts are placed directly, or after being treated in various ways, on the granulating surface in rows at uniform intervals. Then the entire wound surface is covered with a piece of wide-meshed gauze, which extends beyond the edge of the wound, and on top of this "safety net" that serves to keep the grafts in place when the bandage is changed is placed a piece of 4-ply linen, cut to fit precisely the form of the wound and moistened with a 2 % boric acid solution in 2 ‰ mucilago agar covered by cambric. The bandage is changed 4-6 times a day, depending on the secretion. After 48 hours the "safety net" is removed. As a rule the grafts will then be firmly attached and the bandaging treatment is continued. In 3-4 days new-formed epidermis may be seen to extend from the margin of the grafts, an this new-formed epidermis keeps on growing during the following days, forming rows of epidermic islands of the same size which gradually merge and extend to the margin of the wound, which finally thus will be covered by a continuous layer of epidermis. In most cases the grafts from the receptors and from the donors behave perfectly alike, provided they have not been exposed to

different agents. Furthermore, in most cases the healing of the grafts appears to be definitive. Thus my material contains several cases with an observation period of from 6 months to 1 year in which there was at no time any sign of detachment of the grafts. It is very likely, however, that in the primarily healing donor grafts the cells gradually degenerate and are replaced by cells from the receptor, but such a late change will presumably play no role in the experiments that are to be mentioned in the following.

In some cases, however, the course of the grafting has been different. For one thing, it may happen that both the receptor and the donor grafts are detached within the first week after the transplantation, presumably because the granulation tissue has been unsuitable to the grafts. Further, sometimes—most often after a couple of weeks—the donor grafts become detached in toto whereas the receptor grafts keep on growing. More frequently, however, we meet with the interesting phenomenon that the center of the donor grafts—*i.e.*, the original transplants—become detached, while the surrounding ring of new-formed epidermis keeps on growing. Once the same phenomenon has been observed also in receptor grafts simultaneously with the corresponding process in the donor grafts.

When thus it has been possible with the given technique to obtain at any rate apparently lasting takes of homologous skin grafts in most of the cases, this may presumably be due to various circumstances. In the first place, very small skin grafts will presumably be better off with regard to rapid vascularization than larger flaps. Furthermore, the change of bandage through the "safety net" offers the advantage that the wound secretion is removed continuously without the grafts having any occasion to become detached during the first critical days. Finally, with employment of this method, new-formed "embryonal" epidermis grows rapidly from the edges of the grafts, and within a short time it will far surpass the originally transplanted epidermis in amount. Very likely this new-formed epidermis is less "specific", more readily compatible with the host organism than the original. This is indicated, among other things, by the above-mentioned

observations where the original transplant became detached, whereas the surrounding ring of new-formed epidermis kept growing. In the present work I shall leave out of consideration the many other interesting biological conditions which this technique makes it possible to investigate more thoroughly, and which for instance are dealt with in the interesting experiments of *P. B.*

Figure 1.

The transplantation technique employed. 4 rows of small "pinch grafts" in beginning uniform growth on the 3' day after the transplantation, immediately before removal of the "safety net".

Medawar,[3] keeping only to the possibilities which it affords for investigation of allergic-eczematous hypersensitiveness. As will be noticed, this technique allows of "growing" epidermis from patients with an allergic eczematous hypersensitivity on receptors and comparing the growth of these grafts and their reaction to various agents with corresponding control grafts from the receptor himself. As a matter of fact, it is a sort of "tissue culture" on a living medium we are working with here, and experience has shown that under uniform conditions the receptor and donor grafts usually behave alike, *i.e.* heal and keep growing in the same manner.

[3] Br. j. exp. path. 27: 9 & 15, 1946 and earlier publications.

As is well known—*e.g.* in patch tests—it is possible with a specific allergen to produce an eczematous reaction in a patient who is hypersensitive to the substance concerned. Generally this reaction has been interpreted as a primary damage to the epidermis, and thus it was to be expected that an injury of this kind would manifest itself in the healing tendency or growth of the transplanted skin pieces. The given technique allows us to investigate this matter by simultaneous transplantation of donor and receptor grafts and exposure of these grafts either before the transplantation or after the healing to the allergens specific of the donor. On the other hand, we may hardly expect to see any true eczematous reaction in the grafts even though the conditions for such a specific influence be present. For, in its structure the new-formed epidermis of the grafts deviates essentially from the normal cornified epidermis, and evidently it is incapable of producing a typical eczema reaction. This is evident, among other things, from experiments with patch tests at the margin of healing wounds in allergics, where only the normal skin surrounding the wound will show the typical eczema reaction, whereas no characteristic changes will appear in the border of epidermis along the margin of the wound.

The experiments have been carried out according to the following plan:

A. Transplantation from eczematous allergic donor to normal receptor, with control grafts from the receptor himself. After taking, exposure of donor and control grafts to the allergen specific to the donor.

B. Transplantation from normal donor to allergic eczematous receptor, with control grafts from the receptor himself. After taking, exposure of the donor and receptor grafts to the allergen specific to the receptor.

C. Transplantation of grafts from eczematous allergic donor after preceding exposure of the grafts to the specific allergen.

Experimental Group A.

1. Receptor: E. P., ulcer of the leg. Donor: K. R., allergy to mercury.

9/5/46: Transplantation of 7 receptor and 11 donor grafts.
14/5/46: All grafts in good and uniform growth.
22/5/46: Compress with 1 ‰ $HgCl_2$ for 24 hours.
23/5/46 and 24/5: No visible reaction. Grafts growing uniformly.
28/5/46: Grafts all healed.

2. Receptor: H. J., ulcer after freezing with CO_2.
Donor: K. R., allergy to mercury.
23/5/46: Transplantation of 5 receptor and 10 donor grafts.
26/5/46: Uniform growth of all grafts. Dressing with 1 ‰ $HgCl_2$ for 24 hours.
27/5/46: No visible reaction.
During the following weeks all the grafts healed with uniform growth.

3. Receptor: F. R., burn. Donor: K. R., allergy to mercury.
23/5/46: Transplantation of 6 receptor and 9 donor grafts.
27/5/46: Uniform growth of all grafts.
28/5/46: Dressing with 1 ‰ $HgCl_2$ for 48 hours.
1/6/46: Apparently growth arrested in all grafts.
3/6/46: Growth again visible of donor as well as receptor grafts.
9/9/46: Grafts covering the entire wound surface.

4. Receptor: A. R., burn. Donor: a) D. H., allergy to mercury; b) S. L., allergy to mercury.
4/6: Transplantation of 10 receptor grafts, 10 grafts from a) and 10 from b).
12/6: a) and b) grafts growing more slowly than receptor grafts. Dressing with 1 ‰ $HgCl_2$ for 24 hours.
13/6: Redness and swelling under the a) and b) grafts.
15/6: a) and b) grafts about loosening.
17/6: a) and b) grafts detached.

5. Receptor: A. R., burn. Donor: F. B., lucosil[3] allergy.
19/6: Transplantation of 8 receptor and 11 donor grafts.
24/6: All grafts growing poorly. Dressing with 1 % lucosil for 24 hours.
25/6: No distinct changes.
27/6: Some grafts detached in both groups. The remaining receptor and donor grafts grow uniformly.
Subsequent course: Uniform growth and healing.
9/9: Solid scar formation.

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[3] Lucosil = A sulphonamide derivative.

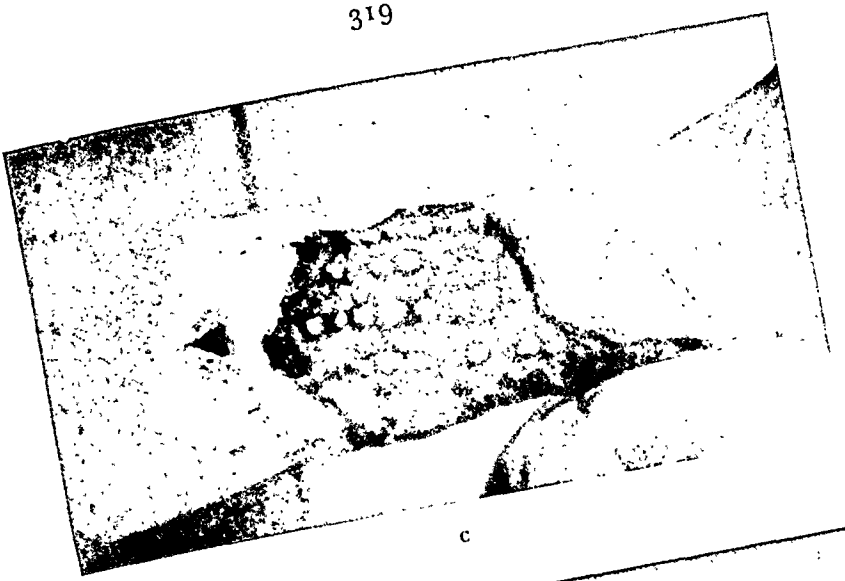
a

b

Figure 2.

Transplantation to normal receptor of grafts from her own skin (the two outer rows) and grafts from a patient with primula allergy (the two middle rows).

a) 6' day after the transplantation, immediately before dressing with primula extract for 24 hours.
b) 10' day. Uniform growth of donor and receptor grafts.
c) 14' day. Uniform growth.
d) 18' day. Uniform growth.



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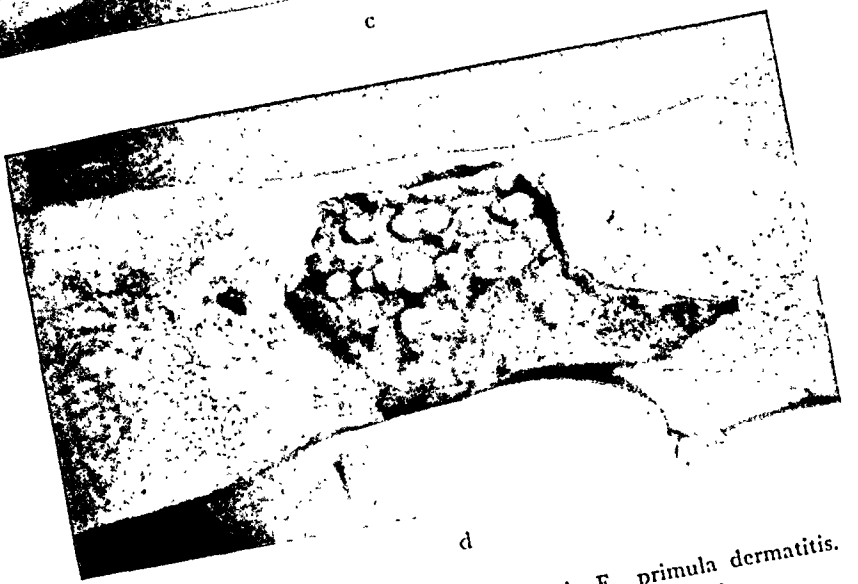


d

6. Receptor: A. J., varicose ulcer. Donor: A. E., primula dermatitis.
21/6: Transplantation of 16 receptor and 16 donor grafts.
24/6: Uniform appearance of grafts.
27/6: A few donor grafts detached. Dressing with primula extract for 8 hours.
28/6: No visible reaction.
1/7: Uniform growth of donor and receptor grafts.
Subsequent course: Uniform growth and healing.
2/9: Solid scar formation.

7. Receptor: M. J., varicose ulcer. Donor: M. L., lucosil allergy.
24/8: Transplantation of 6 receptor and 6 donor grafts.
2/9: Uniform growth of grafts. Dressing with lucosil ointment for 24 hours.
3/9: No visible reaction.
Subsequent course: Uniform growth and healing.

8. Receptor: H. C. J., ulcer after freezing with CO_2. Donor: S. R., "brunisol"[4] allergy.
30/8: Transplantation of 19 receptor and 10 donor grafts.
4/9: Uniform growth of all grafts. Dressing with 2 % "brunisol"[4] for 24 hours.
6/6: No visible effect.
9/9: Good and uniform growth of all grafts.
28/9: Healing with solid scar formation.

9. Receptor: M. J., varicose ulcer. Donor: T. A., terpentine allergy.
12/9: Transplantation of 5 receptor and 5 donor grafts.
17/9: Uniform growth of grafts. Dressing with 10 % terpentine in oil for 24 hours.
18/9: Slight irritation of all grafts and ulcer.
21/9: Uniform growth of receptor and donor grafts.
29/9: Grafts covering the entire wound surface.
5/10: Bulla formation with detachment of the donor grafts.

10. Receptor: V. J., varicose ulcer. Donor: A. K., lucosil allergy.
21/11: Transplantation of 5 receptor and 5 donor grafts. Dressing at once with lucosil ointment for 24 hours, then with boric acid-agar.
26/11: All grafts growing, receptor grafts a little more vigorously than donor grafts.
28/11: Now uniform growth. Again dressing with lucosil ointment for 24 hours.
29/11: No visible effect.
5/12: Uniform growth.
20/12: Detachment of all grafts, commencing in the center.

Of the 10 experiments above, for study of the effect of the specific substance on skin grafts from patients with eczematous allergy *after* transplantation to a non-allergic individual, 8 turned out completely negative, as it was not possible to demonstrate any visible changes in the grafts, which grew just as well as the grafts from the receptor himself. Only in Exp. 4 did the donor grafts

[4] Brunisol = A paraphenylendiamine derivative.

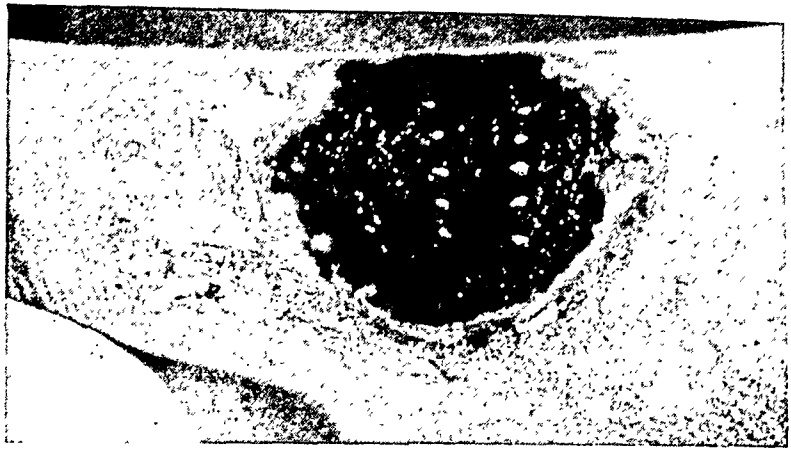
become detached early (after 9 days). But, as these grafts showed a poorer growth than the controls even before application of the specific substance, it does not seem quite warrantable to conclude that their detachment was due to this exposure. In Exp. 10 the grafts were exposed to the action of the specific substance immediately after the transplantation, and here a transitory arrest of growth was observed, which was eliminated during the following days, so that at any rate there was no distinct effect from the exposure.

So the conclusion will be that specific eczematous agents generally will produce no visible changes in grafts from patients with corresponding allergic reactions, when these grafts are growing on a wound surface in a non-allergic individual. As a rule it is also impracticable to demonstrate any effect upon the rate of growth. In so far as any specific process whatever takes place under treatment of this kind, then, it seems to do no visible damage to the vitality of the epidermis.

Experimental Group B.

This group comprises patients with varicose ulcer and eczematous-allergic hypersensitiveness—most often after some previous treatment. So, in contrast to group A the receptors here are allergic, while the donors have been non-allergic or allergic to some other substance than the receptors. While group A afforded a possibility of looking into the question whether any specific reactivity be present in the transplant, group B gives us a chance to see whether reactivity may perhaps be transmitted from the already allergic host to the non-allergic graft.

11. Receptor: A. J., varicose ulcer, allergy to dinitrochlorbenzene.
Donor: I. J., benzocaine allergy.
20/8: Transplantation of 10 receptor and 10 donor grafts in two longitudinal rows.
27/8: Uniform growth of all the grafts. Dressing of the upper half with 2 % benzocaine-solution of the lower half with ½ ‰ dinitrochlorbenzene-solution.
28/8: No noticeable difference between the grafts.
2/9: Uniform growth. Dressing of the upper half with 10 % benzo-



a

b

caine ointment, of the lower half with 2 % dinitrochlorbenzene in oil.

3/9: Lower half of the grafts looking necrotic, equally in both groups. Upper half, no visible changes.

5/9: Partial detachment of the lower grafts, uniformly for the two groups.

16/9: Some of the lower grafts have regenerated.

12. Receptor: T. A., varicose ulcer, terpentine allergy. Donor: N. J., normal.

12/9: Transplantation of 5 receptor and 5 donor grafts.

c

Figure 3.

Transplantation to normal receptor of grafts from her own skin (upper row) and grafts from a patient with lucosil allergy (lower row).

a) 3' day after transplantation, immediately after which dressing with lucosil ointment for 24 hours.

b) 7' day. Donor and receptor grafts in good and uniform growth. Lucosil treatment renewed.

c) 14' day. Uniform growth.

16/9: Uniform growth of grafts. Dressing with 5 % terpentine in oil for 24 hours.

17/9: No visible effect on the grafts.

19/9: Uniform growth of grafts.

23/9: Uniform growth.

13. Receptor: S. N., varicose ulcer, allergy to mercury. Donor: P. H., quinine allergy.

28/10: Transplantation of 10 receptor and 10 donor grafts in two rows.

1/11: Uniform growth of all the grafts. Dressing of upper half with ½ ‰ $HgCl_2$, of lower half with ½ % quinine chloride for 24 hours.

2/11: No noticeable difference between the two rows of grafts.

6/11: In the lower half of the donor and receptor rows, one graft has become detached in each row.

8/11: Uniform growth of the grafts. Repetition of the treatment as on 1/11.

11/11: No difference in the growth of the grafts.

a

b

14. Receptor: A. K., varicose ulcer. Terpentine and lucosil allergy. Donor: V. J., normal.

21/11: Transplantation of 5 receptor and 5 donor grafts. Dressing at once with lucosil ointment.

22/11: Pronounced purulent secretion. Discontinuance of lucosil ointment. Application of boric acid-mucilago agar.

26/11: Good growth of grafts, that of the donor grafts a little more vigorous.

28/11: Rather uniform growth of grafts. Repetition of treatment as on 21/11.

c

Figure 4.

Transplantation to receptor with mercuric allergy of grafts from his own skin (the two middle rows) and donor grafts from patient with quinine allergy (the two outer rows).

a) 4' day after transplantation, immediately before dressing: with ½ % quinine chloride on lower half of the ulcer, with ½ ‰ mercuric chloride on the upper half.

b) 9' day. Uniform good growth of most of the grafts. In each of the two lower rows, however, one graft has become detached.

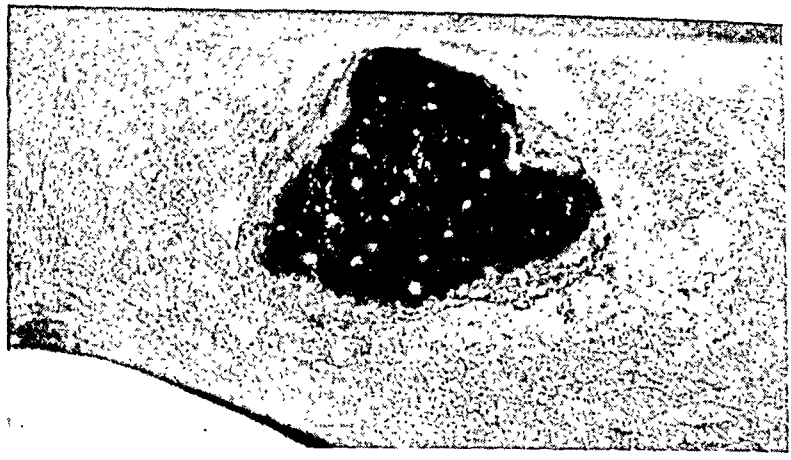
c) 16' day. Uniform growth. (Treatment with quinine and mercuric chloride as above was repeated on the 11' day).

29/11: No noticeable effect.
5/12: Uniform growth of grafts.
7/12: Detachment of the donor grafts.

The results obtained in group B show that there is no noticeable effect on the growth of the grafts from the given treatment and no distinct difference between donor and receptor grafts when they are grown on the wound surface of an allergic and exposed to the substance to which the receptor is hypersensitive.

Experimental Group C.

The purpose of the experiments in this group was to investigate whether a skin area which already had been exposed to the action



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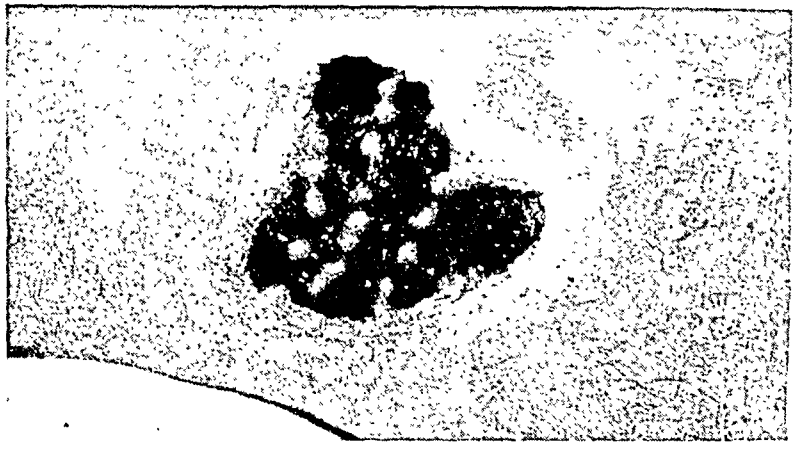

b

of a specific eczematous-allergic factor, after transplantation might behave differently from normal skin. First an experiment was made in which grafts from a patient with eczematous allergy were placed in a solution of the specific allergen for 1-6 hours, and then they were transferred to the wound surface. The controls were receptor grafts treated in the same way, besides donor and receptor grafts that had been placed in saline for the same length

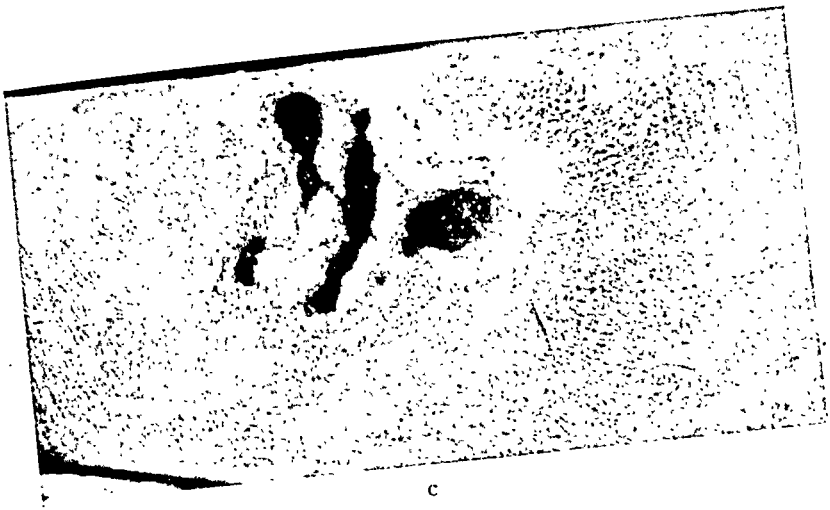

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Figure 5.

Transplantation to receptor with lucosil allergy of grafts from her own normal skin (top row), grafts from an area of her own skin immediately after electrophoretic treatment with the allergen (bottom row), and grafts from an area of her own skin showing a strong eczematous reaction 3 days after electrophoretic treatment with the allergen (middle row).

a) 8' day after the transplantation. Uniform growth of all the grafts.
b) 11' day. Uniform growth.
c) 16' day. Uniform growth and commencing healing.

of time. It was found, however, that staying in saline even for only a few hours lowered the vitality of the grafts noticeably. So, presumably, this source of error may render the method less serviceable for examination of a possible specific effect of the allergen. Transplantation from positive patch tests involves the drawback that the fully developed eczematous process with vesiculation, etc., complicates the conditions, as the exudative processes might damage the epidermic cells secondarily.

These difficulties are avoided by taking the grafts from skin areas exposed to the action of the specific substance through *electrophoresis*. In this way the grafts can be transplanted immediately after the exposure, before the skin presents any noticeable changes, while the epidermis has already become the site of the effect which 24 hours later will result in an eczematous eruption.

Besides, of course, grafts may be transplanted also at later stages, so that the influences of the exudative processes themselves may be studied too.

This group comprises 5 experiments. In the first two electrophoretically treated skin was transplanted together with control grafts of untreated skin from an allergic to a normal receptor, and with receptor grafts for comparison. The third experiment was carried out on an allergic receptor, on whom autotransplantation was performed after electrophoresis. In the last two experiments grafts were transplanted from freshly eczematous areas produced by ordinary patch tests.

The experiments were carried out as follows:

15. Receptor: D. J., varicose ulcer. Donor: S. N., mercuric allergy.
28/11: Transplantation of 5 receptor and 10 donor grafts. Of the donor grafts 5 were taken from normal skin, 5 from skin which 2 hours previously had been treated electrophoretically with 1 ‰ $HgCl_2$. On the following day, the treated skin area showed strong eczematous reaction.
2/12: 2 "electrophoretic grafts" detached. Good and uniform growth of the remaining grafts.
5/12: Uniform growth of all the remaining grafts.
12/12: All the remaining grafts growing uniformly.

16. Receptor: A. O., varicose ulcer. Donor: A. R., nickel allergy.
4/12: Transplantation of 7 receptor and 10 donor grafts. 5 donor grafts were taken from normal skin, 5 from an area which 1 hour before had been treated electrophoretically with 1 % $NiCl_2{,}6H_2O$. On the following day the treated skin areas showed a strong eczematous reaction.
11/12: All grafts growing, the receptor grafts not quite as vigorously as the two other groups.
13/12: Uniform growth of all the grafts.
23/12: All the grafts healed.
27/12: Detachment of the donor grafts.

17. Receptor and donor: D. J., varicose ulcer, lucosil allergy.
1/7: Transplantation of 1) 8 grafts from normal skin; 2) 8 grafts from skin which 3 days before has been treated electrophoretically with lucosil and now was the site of a strong eczematous reaction; 3) 8 grafts from a skin area which immediately before had been

treated electrophoretically with lucosil and on the following day presented a strong eczematous reaction.

4/7: Poor growth of all the grafts.

7/7: A few grafts in group 3) detached; also poor growth in groups 1) and 2).

10/7: Now good growth of all the remaining grafts. No difference between the groups.

15/7: Continuously good and uniform growth.

28/7: All grafts healed.

18. Receptor: E. N., varicose ulcer. Donor: D. J., chromate allergy.

14/12: 5 donor grafts from skin area with beginning eczematous reaction after patch test with 1 ‰ potassium bichromate. 5 donor grafts from normal skin. 5 receptor grafts.

18/12: All grafts growing. The bichromate-treated donor grafts grow a little less vigorously than the others.

23/12: All grafts growing, the bichromate grafts still lagging a little behind the others.

27/12: Now almost uniform growth of all the grafts.

19. Receptor: A. O., varicose ulcer. Donor: B. R., coumarin allergy.

10/12: Transplantation of 1) 5 grafts from donor skin with commencing eczematous reaction after patch test with 5 % coumarin in oil; 2) 5 grafts from untreated donor skin; 3) 5 grafts from receptor after patch test with 5 % coumarin in oil, which did not elicit any reaction; 4) 5 grafts from untreated receptor skin.

16/12: Uniform growth of all the grafts.

23/12: Still uniform growth of all the grafts.

The experiments in group C, with exposure of the grafts to the specific agent *prior to* their transplantation, have given a similar negative result as was obtained in the experiments with exposure of the grafts to the specific agent *after* the transplantation (groups A and B). On iontophoretic introduction of the specific agent in the skin of the hypersensitive donor, the epidermis appears not to have been damaged decisively, as the growth of grafts from this area does not deviate from that of donor grafts of normal skin or receptor grafts. Even on transplantation from a fully developed eczematous reaction (Exp.s 17 and 19) the vitality of the epidermis appears to be well preserved. Only in Exp. 18 the eczematous changes appear to have brought about a somewhat lower rate of growth as compared to that of the controls.

CONCLUSION and SUMMARY

By means of a special grafting method it is practicable to transplant small pieces of skin from one individual to a granulating wound surface—*e.g.* a varicose ulcer— in another individual and obtain uniform growth of these donor grafts and grafts taken from the receptor himself—at any rate for a couple of weeks. Grafting from allergics to normal subjects, and from normal subjects to allergics, and exposing of the donor grafts plus the control grafts from the receptor to the action of the specific agent affords a possibility of investigating whether a specific reactivity is connected with the skin itself (grafting from allergic to normal subject) or with "humoral" factors (grafting from normal subject to allergic). Unfortunately the conditions in new-formed growing epidermis differ so much from the conditions in normal skin that an eczematous reaction is not to be expected after exposure to the specific allergic agent. These experiments, therefore, cannot give any information about the influence of the various conditions upon the very features of the allergic *eczematous* reactions but may merely elucidate the possible noxious effect on the *vitality* of the epidermic cells under the different experimental conditions.

From the experiments it is evident that exposure to the specific allergic substance of eczematous allergic skin growing on a wound surface in a non-allergic subject does not give rise to visible changes or inhibition of growth. Nor does normal skin grafted on a wound surface in an allergic and then exposed to the action of the allergen show any specific changes. Not even grafts from the allergic's own skin show any inhibition of growth after specific exposure even though all conditions for the appearance of a reaction here would be expected to be present. Electrophoretically exposed allergic skin shows no inhibition of growth as compared with untreated skin on transplantation even though the grafting is carried out as autotransplantation in a state of fully developed eczema. Grafting from eczematous skin after ordinary patch tests was carried out in two cases, and only one of them showed some inhibitional growth.

It seems reasonable, then, to interpret these results to the effect

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(Institut de Clinique et de Policlinique Médicales, Prof. L. Brull, Université de Liége).

HEPATOTHERAPIE INJECTABLE, INSULINOTHERAPIE ET MANIFESTATIONS ALLERGIQUES[1]

Par

G. BARAC

Un homme de 60 ans, est adressé à la Clinique par son médecin qui nous fait savoir que le malade, atteint d'anémie pernicieuse, à été traité à différentes reprises par des injections. I. M. d'extrait de foie, avec résultats satisfaisants, mais de courte durée. Avant son admission actuelle dans le Service, ce malade a reçu une nouvelle série d'injections d'une préparation hépatique, dont les dernières furent très mal supportées.

Comme nous sommes précisément en train d'étudier l'effet antianémique d'une nouvelle préparation de foie injectable, nous croyons tout indiqué son essai chez notre malade. Dès la première injection I. M. de 2 cc du nouvel extrait, le malade manifeste, sitôt la piqûre terminée, une intolérance marquée, caractérisée par de la pâleur brusque, vomissements, pouls imperceptible, chute et perte de conscience. Heureusement, ce choc est de courte durée. Une injection I. M. de 5 cc de gluconate calcique, faite avant celle du nouvel extrait, permet dès le lendemain, d'éviter ce choc et de poursuivre le traitement pendant 11 jours. Malgré cette médication, non seulement il n'y a aucune amélioration sanguine, mais l'état s'aggrave considérablement. Nous administrons du protoxalate de fer à la dose de 2 grs. par jour et, 6 semaines après ce traitement, l'hémoglobine passe de 22 à 72 % et les hématies de 1.000.000 à 3.000.000. Le malade quitte le Service fortement amélioré, après avoir gagné 7,5 kgrs.

[1] Conférence faite devant la Société Belge de l'Allergie le 27 juin 1948.

Ce malade revient 7 semaines après et déclare que, durant un mois, son état s'était maintenu bon. Bientôt apparaissent des nausées fréquentes, l'appétit diminue, le malade maigrit de 3,3 kgrs.

A l'entrée, la situation hématologique est sérieuse, hématies 1.650.000 hémoglobine 37 %. Les globules rouges sont altérés. Il y a 2 % de normoblastes. Cette-fois-ci, comme la première fois d'ailleurs, il y a achlorhydrie, cependant qu'on nouvel examen radiologique permet d'éliminer la néoplasie gastrique.

Très rapidement l'état s'aggrave. L'appétit est nul. Le malade se plaint de céphalées violentes. Il vomit. Sa température est à 39° C. A deux jours d'intervalle, on pratique 2 transfusions, chacune de 600 cc de sang frais, bien tolérées. Pendant 2 semaines, on donne une potion acide, du protoxalate de fer à raison de 1,5 gr. par jour, et l'on injecte journellement en I. M. des extraits hépatiques de 2 marques différentes. Cette-fois-ci les extraits hépatiques sont bien tolérés, mais le résultat thérapeutique de tous nos efforts est nul. Le malade est mourant.

Nous avons vu à la policlinique, voici trois jours, une femme de 68 ans, anémique, qui a reçu à plusieurs reprises des extraits hépatiques injectables. Comme dans le premier cas, ce traitement fut d'abord bien toléré, mais ne l'est plus depuis quelques mois. En effet, l'injection de 2 préparations hépatiques différentes, même en toutes petites quantités, a déterminé il y a quelque temps des manifestations de « véritable maladie sérique ».[1] Son médecin, de qui nous tenons ces derniers renseignements, n'ose plus la traiter de la sorte et au moment où nous voyons la malade, elle est presque totalement impotante des membres inférieurs, cette impotance ayant débuté vioci 4 mois et s'accentuant depuis. Je ne sais pas encore ce que nous pourrons faire pour elle, mais le cas est grave et il l'est certainement, en partie tout au moins, à cause de l'intolérance qu'elle semble avoir développée vis-à-vis des injections d'extraits de foie, intolérance qui interdit désormais ce mode de traitement.

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[1] 0,2 cc. de Pernaemon en intra-termique ont provoqué chez elle, sous nos yeux, un choc intense ; au même moment, la réaction générale et locale fut négative chez deux témoins

Une femme de 63 ans est hospitalisée pour bronchectasie bilatérale. Elle tousse et émet environ 100 cc de crachats non bacillifères par jour. Comme l'état général est mauvais, puisque pour 1 m. 63 notre malade pèse 42 kgrs., comme, d'autre part, elle mange peu, nous lui injectons de l'insuline ordinaire 2 × 10 U. par jour, pendant 7 jours, puis 3 × 10 U. par jour, ceci dans le but d'augmenter son appétit. L'état général s'améliore, la malade gagne du poids, sa température tend à revenir à la normale, son expectoration tombe à 40 cc par jour. Mais voici que 11 jours après le début du traitement insulinique, des rougeurs commencent à apparaître aux endroits d'injections de l'insuline. Encore 2 jours de traitement insulinique et les rougeurs deviennent prurigineuses. Nous interrompons l'administration d'insuline pendant 24 heures. Le lendemain matin, on injecte 10 unités d'insuline ordinaire, comme toujours en sous-cutanée. Dans la soirée, apparaît une petite plaque urticarienne autour d'une malléole. Le jour suivant, la malade signale avoir passé une très mauvaise nuit. A l'examen, on note une urticaire généraliséetrès prurigineuse. La face est rouge et fortement œdématiée. Devant cette situation, nous sommes obligés d'interrompre les injections d'insuline.

Une femme de 71 ans, chez qui l'on découvre pour la première fois un diabète, est admise à la Clinique, pour équilibration. Comme son poids est suffisant, malgré l'amaigrissement récent, comme d'autre part, il s'agit d'une personne âgée dont l'activité physique est très légère, on lui prescrit un régime comportant 135 gr. de glucides et 1700 calories en tout. D'autre part, on lui injecte d'abord 2 × 30 unités d'insuline ordinaire par jour. La glycosurie disparaît en trois jours et la malade rentre chez elle. Elle revient trois semaines après, déclarant que depuis quelques jours les injections d'insuline provoquent localement des rougeurs insupportables. Dans le Service on constate que dès la première injection d'insuline apparaît localement, pour s'étendre sur une zone d'une certaine importance, un érythème, prenant bientôt l'aspect d'une urticaire très prurigineuse.

Je pourrais, certes, vous raconter d'autres observations encore, mais ceci n'est guère indispensable et il me paraît préférable de

discuter maintenant quelques questions en rapport avec ces diverses observations, questions intéressantes au point de vue scientifique et pratique.

Et tout d'abord, les manifestations allergiques que nous étudions sont-elles exceptionnelles ?

Voyons premièrement la question de l'hépatothérapie injectable. Au début de cette médication, les réactions allergiques étaient très fréquentes et, sans doute, provoquées par les impuretés protéiques des préparations commerciales. Avec le temps, les firmes, informées de cest inconvénient, se mirent à purifier davantage leurs préparations et les nouveaux extraits devinrent certainement plus tolérables quoique pas toujours plus actifs sur l'hématopoïèse, au contraire. Cependant, les manifestations allergiques ne disparurent pas totalement. Dans le Service on a vu une malade chez laquelle des injections d'extrait de foie déterminaient une vasodilatation considérable de la face des avant-bras et des jambes, cette vasodilatation pouvant durer jusque 3 heures. Chez une autre, chaque injection s'accompagnait de forte hyperthermie avec rougeur de la face. Une autre encore, présentait dans les mêmes conditions, endehors d'une vasodilatation faciale intense, une sensation de froid dans tout le corps et des céphalées suivies d'abattement et d'anorexie. Une éruption urticarienne avec œdème des paupières a également été constatée chez une malade soumise à la même médication. La littérature des ces dernières années contiënt, d'autre part, la description de cas d'asthme, d'urticaire, de dyspnée, d'œdème angio-neurotique, de vomissements apparus au cours du traitement par foie injectable.

Faut-il mettre ces manifestations sur le compte d'impuretés protéiques présentes dans les extraits hépatiques ? Une pareille explication paraît à première vue logique. Mais s'il en est ainsi, pourquoi ces manifestations n'existent-elles pas à la longue chez tous ceux qui reçoivent des extraits hépatiques ? Pourquoi, d'autre part, le même produit peut-il déterminer chez telmalade des phénomènes d'intolérance locale ou générale ou les deux à la fois, alors qu'à la même époque, d'autres malades qui reçoivent le même produit le supportent bien ? Pourquoi aussi, le même malade, et ce fut le cas du nôtre, présente-t-il vis-à-vis de la même

préparation hépatique d'abord des phénomènes de véritable choc, puis quelque temps après, une tolérance apparamment satisfaisante ?

Pourquoi enfin, un anémique Biermérien répond-il d'abord bien au traitement hépato-thérapique injectable, auquel il devient tout à fait réfractaire dans la suite ?

Pour les trois premières questions on peut évidemment dire qu'il s'agit d'un ou plusieurs facteurs individuels, invoquer le terrain, ce qui est une constatation empirique, mais nullement une explication. Pour la dernère question, on peut tout au moins émettre une hypothèse. C'est ce que nous avons fait en 1939. Je tiens à préciser que le seul intérêt de cette hypothèse était et est toujours d'indiques une voie possible à la recherche dans ce domaine.

Voici en quoi elle consiste : l'administration prolongée d'extraits hépatiques injectables détermine dans des conditions qu'il reste à rechecher l'apparition d'une anti-hormone spécifique.

Ceci m'amène à vous rappeler quelques notions concernant les anti-hormones.

C'est *Collip* qui fut le premier à formuler la notion des anti-hormones en 1933. Cet auteur a constaté avec *Anderson* que l'administration à des Rats d'un extrait contenant de l'hormone thyréotrope provoque chez ces animaux une augmentation du métabolisme basal. Lorsque ce traitement est suivi pendant quelque temps, le métabolisme basal non seulement revient à la normale, mais baisse, au contraire, d'une façon prononcée, atteignant bientôt des valeurs notées seulement chez des animaux hypophysectomisés. Mais, fait plus important encore, le sérum des Rats ainsi traités, injecté à d'autres animaux, s'oppose chez ceux-ci à toute élévation du métabolisme basal, que provoquerait sinon l'administration d'hormone thyréotrope. Le sang des animaux rendus résistants envers l'hormone thyréotrope, renferme donc une substance qui inhibe l'activité de cette hormone. Comme vous le savez, d'autres actions anti-hormonales furent décrites plus tard, en cas d'administration prolongée d'hormones gonadotropes, d'hormone de croissance, d'hormone cétogène, etc.

Est-il possible d'admettre une pareille action anti-hormonale dans le cas du facteur anti-pernicieux du foie ?

En principe rien ne s'y oppose. Mais alors qu'à l'époque où nous avons émis cette hypothèse, des recherches expérimentales dans ce domaine étaient impossibles, parce que l'on ne disposait pas alors d'une hormone anti-anémique d'origine hépatique, chimiquement pure, il n'en est plus de même aujourd-hui. En effet, les laboratoires *Merck* de Rahway ont isolé tout récemment, à partir du foie, une substance chimiquement encore indéterminée bien que déjà obtenue à l'état cristallisé, qui serait, semble-t-il, le vrai facteur anti-pernicieux hépatique. Cette substance, à la dose de quelques gammas, s'est montrée nettement active dans l'anémie Biermérienne.

Grâce à cette nouvelle substance, il sera possible d'aborder des recherches sur la question de savoir si oui et alors dans quelles circonstances, elle peut devenir antigénique et former un anticorps ou si vous voulez une anti-hormone spécifique.

Nous pouvons maintenant passer au problème des *manifestations allergiques à l'insuline*. L'examen de de cet important problème nous retiendra davantage pour les raisons suivantes :

1) Le diabète est *socialement* important en raison du grand nombre de malades qui en sont atteints et de la chronicité de cette affection.
2) Le traitement rationnel du diabète vise non seulement au rétablissement de l'equilibre rompu par la viciation du métabolisme hydrocarboné, mais a également pour but de permettre au diabétique une existence professionnelle normale. Or, ceci implique une plus large utilisation d'insuline.
3) Le fait que l'insuline a pu être préparée à l'état *cristallisé*, a déjà permis une série d'observations des plus intéressantes dans le domaine qui nous préoccupe.
4) La réalisation des diverses insulines retard, la synthèse chimique d'une série de dérivés de cette hormone, ont conduit à des recherches importantes relatives à ses propriétés antigéniques.
5) Certaines techniques de laboratoire que nous aurons l'occasion

de citer au cours de cet exposé, ont apporté des données très instructives sur le comportement antigénique de l'insuline chez les diabétiques.

Comme nous l'avons fait, pour les préparations hépatiques injectables, voyons tout d'abord qu'elle est la fréquence des manifestations allergiques au cours de l'insulinothérapie. Ici aussi, pendant les premières années d'utilisation de l'insuline en clinique, ces manifestations étaient assez fréquentes, en raison vraisemblablement des impuretés protéiques contenues dans les préparations commerciales. Plus tard, la purification toujours plus prononcée de ces préparations a réduit ces manifestations, sans toutefois les supprimer complètement. Le pourcentage des réactions locales varie de 7 à 30, suivant les auteurs. Les réactions générales sont heureusement très rares suivant *Joslin*.

Peut-on attribuer les manifestations allergiques des préparations commerciales d'insuline exclusivement à des traces d'autres protéines qu'elles contiendraient ? Nous l'avons pensé pendant quelque temps. En effet, chez notre diabétique, qui a présenté les réactions locales à l'insuline commerciale, nous avons pu remplacer celle-ci par de l'insuline cristallisée, les solutions à injecter ayant été réalisées chez nous. Ces dernières injections furent très bien tolérées dès le début, sans la moindre réaction locale.

Cependant, cette constatation n'est pas suffisamment probante et nous savons actuellement, par des recherches de plusieurs auteurs, que l'insuline cristallisée peut, elle-aussi, être responsable de manifestations allergiques.

Je dois maintenant préciser deux notions qui n'ont pas toujours été suffisamment distinguées :

1° Les manifestations allergiques locales ou générales à l'insuline considérée comme antigène.

2° La sensibilité ou la résistance à l'insuline envisagée comme hormone régulatrice de la glycémie.

Cette dernière notion m'amène à vous parler de diabète insulino-résistant, état qui peut être considéré en fait comme une des manifestations les plus curieuses, hélas, quelquefois mortelle, de l'allergie insulinique.

Qu'est-ce donc qu'un diabète insulino-résistant ?

Il faut, croyons-nous, d'abord insister sur le fait qu'il existe une pseudo-résistance à l'insuline. En effet, plus d'une fois nous voyons à la consultation spéciale des diabétiques de notre Policlinique, des malades qui reçoivent de fortes doses d'insuline, sans être équilibrés. Admettons ces malades à la Clinique, mettons-les à un régime approprié que nous contrôlerons avec soin, et le diabète s'équilibrera souvent avec la moitié ou le tiers de la dose d'insuline, avérée inefficace lorsque le diabétique était chez lui. C'est qu'avant, il ne respectait pas son régime et que maintenant il est forcé de le faire.

Mais il existe des diabétiques qui nécessitent réellement de fortes doses d'insuline pour leur équilibration et d'autres chez qui même des doses très considérables et une surveillance étroite du régime restent sans effet.

Voici quelques observations personnelles :

Il s'agit d'une femme de 70 ans, diabétique acromégale. Malgré l'administration, en deux fois, de 120 unités d'insuline par jour, moitié ordinaire moitié retard, la glycémie est de 3,9 gr.‰, la glycosurie de 64 gr.‰ un jour, 57 gr.‰ le lendemain. On passe à 160 unités d'insuline par jour, mais la glycosurie reste toujours entre 51 et 65 gr.‰, la glycémie à jeun étant encore élevée : 1,98 gr.‰. On pourrait objecter que cette illustration est mal choisie, puisque l'acromégalie favorise l'hyperglycémie et peut même être, semble-t-il, la cause du diabète. L'expérience clinique apprend que tous les acromégales diabétiques ne se comportent pas de la même façon. Il y a donc autre chose.

Autre exemple : Un homme de 45 ans est tuberculeux et diabétique. Le diabète est reconnu depuis un an environ, la tuberculose depuis 7 mois. A l'entrée dans le Service, la glycémie est de 3,84 gr.‰, la glycosurie de 65 gr.‰. Le traitement insulinique institué consiste en 3 × 30 unités d'insuline par jour. La glycémie diminue peu. Elle est au 5me jour de 2,87 gr.‰ La glycosurie persiste. Et pourtant, le régime comporte seulement 193 gr. d'hydrates de carbone, c'est-à-dire, 3 gr./Kg. de poids corporel, ce qui n'est certainement pas excessif, sartout chez un tuberculeux. Il faut augmenter l'insuline jusques 170 unités par jour pour voir disparaître la glycosurie. En remplaçant quelque temps après l'insuline ordinaire et l'insuline retard par la Diinsuline, il faut encore 120 unités par pour que la glycémie soit de 0,85 gr.‰.

Autre exemple encore : Un diabétique de 48 ans, entre dans le Service pour infection staphylococcique grave, ayant débuté par un furoncle du cou insuffisamment soigné. A l'examen on est frappé par le décollement de la moitié supérieure du revètement cutané dorsal. L'écoulement de pus est

énorme. La situation est très inquiétante. Ce malade fut sauvé, son infection entièrement guérie, mais 290 unités d'insuline furent nécessaires journellement pendant 25 jours, pour obtenir des glycémies à jeun satisfaisantes : 0,99, 1,12, 0,73 gr.‰. Et cependant, le régime alimentaire comportait seulement 235 gr. d'hydrates de carbone. Remarquons qu'après laguérison de l'infection, 50 unités d'insuline par jour étaint suffisantes pour l'équilibration du diabète.

Vous me direz : il y a infection et c'est elle qui est responsable de cette forte quantité d'insuline nécessaire dans ce cas. C'est exact. Le fait reste cependant et demande une explication. Nous y reviendrons.

Mais peut-on considérer nos malades comme insulino-résistants ? En posant cette question, je ne m'écarte pas du sujet de cette causerie, comme vous allez le voir.

Rooth a donné la définition suivante de l'insulino-résistance : Considérant hypothétiquement que la sécrétion physiologique d'insuline est chez l'homme d'environ 200 unités par 24 heures, cet auteur admet que lorsque l'équilibration d'un diabétique demande plus de 200 unités d'insuline par jour, il s'agit d'un diabète insulino-résistant.

S'il en est ainsi, nos deux premiers cas sont proches de cet état, notre troisième est certainement insulino-résistant.

Toutefois, si l'on tient compte de l'observation de *Mc Clure,* où après ablation du pancréas chez un non-diabétique, le besoin en insuline était seulement de 26 unités par jour, si l'on prend en considération l'observation de *Brunschwig* et collaborateurs, où après pancréatectomie totale chez un diabétique, 40 unités d'insuline par jour étaient suffisantes, le régime alimentaire comportant cependant 400 gr. d'hydrates de carbone, on doit admettre que de nombreux diabétiques qui demandent, disons, plus de 50 unités d'insuline par jour pour leur équilibration, sont déjà insulino-résistants.

Certes, pancréatectomie totale ne signifie pas nécessairement carence totale en insuline endogène, puisque l'on sait, que des îlots pancréatique secondaires en sont pas rares chez l'homme.

A notre avis, pour savoir à partir de quel besoin en insuline un diabète peut être qualifié d'insulino-résistant, on devrait tout

d'abord connaître quel est chez l'homme le débit insulinique normal du pancréas, quelles sont les variations de ce débit sous l'influence de l'alimentation, du travail, etc. quelle est l'importance sécrétoire vicariante des îlots pancréatiques, quelle est l'insulinémie normale, celle que l'on trouve chez les diabétiques non traités, etc. Malheureusement, il n'est pas encore possible de doser l'insuline ssanguine faute de technique, et aussi longtemps que cette impossibilité persistera, les questions soulevées ne seront pas résolues.

Voici enfin, un exemple de grande insulino-résistance mortelle, emprunté à *Felder*. Il s'agit d'une diabétique de 79 ans. Chez cette malade, 50 unités d'insuline cristallisée, injectée en I.V., sont sans effet sur la glycémie. Chez cette malade aussi l'augmentation progressive de l'insuline, administrée en sous-cutanée, jusque 1.100 unités par jour, a été sans aucun effet. La malade est morte dans le coma au 61me jour d'hospitalisation.

Quel est le mécanisme des réactions allergiques à l'insuline ? Quel est le mécanisme de l'insulino-résistance ?

Tuft a décrit, en 1928, le cas d'une diabétique ayant développé des manifestations allergiques locales et générales à l'insuline. Dans ce cas, il lui fut possible de démontrer pour la prémière fois, grâce à la technique de *Praussnitz-Küstner, la présence d'anticorps spécifiques vis-à-vis de l'insuline.*

Lerman, en 1944, rapporte 6 observations personnelles d'insulino-résistance considérable. Deux de ses malades recevaient chacun journellement 2.200 unités d'insuline. Dans ces cas aussi la Cuti-réaction insulinique fut trouvée positive, de même que des précipitines sériques spécifiques à l'insuline cristallisée + Zn. furent mises en évidence. Il est à noter que la disparition de la résistance à l'insuline fut suivie également de celle des anticorps spécifiques.

Lowell a également fait de très intéressantes recherches sur la question de l'insulino-résistance. Voici tout d'abord sa technique : D'après cet auteur, les manifestations hypoglycémiantes insuliniques se manifestent chez la souris par plusieurs symptômes : irritabilité, convulsions, perte de l'équilibre, faiblesse, paralysie des membres, coma. Lorsqu'on injecte à la souris en intra-abdominale 0,5 cc contenant du sérum humain normal et 0,033 unité d'insu-

line, l'effet insulinique est positif chez 80 % des souris. Lorsque cette même solution est enrichie en glucose, de façon à se rapprocher des hyperglycémies diabétiques les plus fréquentes — 2,4 gr. à 3 kr.‰ — le test à la souris est encore positif dans 57 % des case.

Si lors on injecte à la souris du sérum de diabétique insulino-résistant, additionné d'insuline, l'effet insulinique chez la souris est nul. Il redevient chez le même malade positif, lorsque l'insulino-résistance disparaît. Donc, le sérum de malades insulino-résistants contient une substance qui neutralise directement et spontanément l'insuline ajoutée in vitro, au point de supprimer son action hypoglycémiante chez la souris.

Cet auteur note, d'autre part, que dans certains cas l'insulino-résistance se développe vis-à-vis de l'insuline cristallisée de bœuf, par exemple, mais non à l'égard de l'insuline humaine.

Il montre ensuite, qu'après 2 heures de chauffage à 57° C., le sérum de malades insulino-résistants, perd sa propriété de sensibilisation cutanée, alors que son action neutralisante vis-à-vis de l'insuline persiste. Il en conclut que :

1) Allergie cutanée et insulino-résistance sont indépendantes et déterminées par deux facteurs différents.
2) Dans la moelécule d'insuline, agit comme antigène de sensibilisation cutanée un autre groupement chimique que celui responsable de l'action sur le métabolisme du glucose.

Cet auteur a calculé dans un cas d'insulino-résistance que la quantité de plasma en circulation était capable de neutraliser 1.300 unités d'insuline, d'après le test cutané, et 2.000 unités, d'après le test à la souris. Etant donné les techniques utilisées, la concordance de ces deux valeurs doit être considérée comme satisfaisante et, en tout cas, cliniquement très significative. Cette estimation explique comment des doses énormes d'insuline peuvent être supportées par le diabétique insulino-résistant, tout en restant inefficaces quant à la régulation de la glycémie.

D'après *Goldner* et *Rickets,* qui ont étudié 8 cas d'insulino-résistance, la période latente d'apparition des symptômes allergiques à l'insuline varie de 7 à 25 jours. Suivant ces même auteurs, un traitement insulinique discontinu favoriserait les manifestations

allergiques, ce qui cadre bien avec la conception d'anticorps pour l'insuline.

Remarquons encore que dans un cas d'insulino-résistance étudié par *Lowell*, cette résistance se manifestait à l'égard de l'insuline de bœuf, mais non vis-à-vis de l'insuline humaine, cette dernière étant dependant capable de produire des manifestations allergiques locales.

Signalons aussi les très intéressantes recherches de *Harington*. Cet auteur a montré que l'introduction de glucose et de tyrosine dans la molécule d'insuline, confère à celle-ci des propriétés antigéniques. Peut-être cette constatation explique-t-elle, du moins dans certains cas, que la viciation du métabolisme de l'insuline sécrétée, jointe éventuellement à d'autres altérations métaboliques, donne à l'hormone ainsi transformée des propriétés chimiques et immunologiques nouvelles.

Il est, d'autre part, probable qu'en cas de grande infection chez un diabétique, les anticorps formés contre l'agent infectant neutralisent une partie ou la totalité de l'insuline, d'où la nécessité de donner dans ce cas d'assez grandes quantité de cette hormone pour équilibrer le diabète.

En ce qui concerne les manifestations cutanées provoquées par différentes préparations d'insuline, les résudtats de *Page* & *Baumann* sont les suivants :

	Non-allergiques diabétiques reactions cutanées %	Allergiques Non-diabétiques reactions cutanées %
Protamine	48,3	18,5
Globine (bœuf)	2,1	2,4
Globine (humaine) A	0	2,4
Globine (humaine) B	4,2	1,2
Globine bœuf tot.	1,0	1,2
Insuline en milieu chlorhydr.	6,4	1,2
ZnCl 0,02 cc = 4,8 Zn	75,6	41,9
Insuline bœuf (comm.)	3,2	24,6
Insuline cristall.		4,8
Insuline, chlorure de p-azobenzyltriméthylammonium		10,0
Insuline-p. azobenzènesulfonique		3,0

Notons que 4,8 Zn correspondent à 2,4 unités de protamine-insuline-zinc.

Il nous reste un dernier point à considérer.

Que faire en cas d'intolérance ou de résistance aux extraits hépatiques injectables à l'insuline ?

Lorsqu'il s'agit d'hépatorésistance, on peut essayer la thérapeutique pérorale. C'est ce que nous avons fait chez notre anémique pernicieux, en lui donnant de l'Estomon. Le résultat de ce dernier mode de traitement fut dans ce cas vraiment extraordinaire. Le malade dont le pronostic nous paraissait fatal à brève échéance, vit son état s'améliorer en peu de temps, le sang redevenant pratiquement normal. Ce malade a quitté la Clinique en excellent état et nous l'avons suivi pendant des années toujours prenant de l'Estomon et se portant bien. Pour les manifestations d'intolérance locale, on a préconisé la désensibilisation. Je ne l'ai jamais pratiqués, tout ce que je puis vous dire, c'est qu'il faut être prudent. *Hunter* & *Hill* ont peu atténuer et même faire disparaître des réactions allergiques provoquées par l'hépatothérapie injectable, en utilisant un antihistaminique.

Dans les manifestation allergiques locales à l'insuline, on obtient quel quefois de bons résultats en changeant la marque d'insuline utilisée. On peut aussi, d'après *Urbach,* désensibiliser le malade en deux jours par injections alternantes intradermiques, sous-cutanées et intraveineuses d'insuline, en augmentant progressivement les doses utilisées. Ici aussi il faut être très prudent, car dans certains cas la sensibilité cutanée se manifeste déjà pour 0,00001 u. d'insuline, voire même 1/2.000.000 U., d'après *Joslin.*

Collens et collaborateurs signalent avoir obtenu de bons résultats par désensibilisation au moyen de phosphate d'histamine, ce qui est très curieux.

Gastineau et *Leavitt,* cités par *Joslin,* auraient obtenu récemment des résultats favorables par l'emploi de Bénadryl. *Hunter* et *Hill* ont confirmé ces constatations à l'aide d'Anthisan, un autre anti-histaminique.

Le problème thérapeutique de l'insulino-résistance reste entier.

Nous voici arrivés au terme de cette causerie, au cours de laquelle j'ai essayé de vous montrer l'intérêt pratique et scienti-

fique des manifestations allergiques provoquées par l'hépatothérapie injectable et l'insulino-thérapie. Vous avez vu que si dans ce domaine certains phénomènes ont déjà trouvé leur explication, d'autres, bien plus nombreux, attendent leur solution.

A la Clinique Médicale du Professeur *Brull,* où notre Maître a créé depuis longtemps un département spécial pour l'étude du diabète, et où nous disposons d'un riche matériel clinique, nous comptons nous occuper des manifestations allergiques chez les diabétiques.

RÉSUMÉ

L'hépatothérapie injectable et l'insulinothérapie déterminent chez certains malades des manifestation allergiques locales et générales, ainsi que l'apparition d'une résistance à l'égard de ces mêmes traitements. Après une brève description de quelques observations personelles, nous discutons le mécanisme de ces diverses manifestations allergiques, ainsi que le problème de l'hépato et insulino-résistance, en tenant compte de quelques essais personnels et surtout en nous basant sur plusieurs recherches américaines. Nous signalons ensuite les possibilités thérapeutiques actuelles en cas de manifestations allergiques, provoquées par les extraits de foie injectables et l'insuline. En ce qui concerne l'hépato résistance, nous montrons, par un exemple personnel, que la thérapeutique pérorale par extrait gastrique peut être hautement efficace, là où les injections d'extraits hépatiques échouent. Par contre, la grande insulino-résistance ne reconnaît jusqu'à présent, aucune thérapeutique immédiatement active.

SUMMARY

Parenteral therapy by liver-extracts and insulin are the cause, in some patients, of the appearance of local and general symptoms of allergy and that of a resistance to these forms of treatment.

After a brief description of a few personal observations, the author discusses the mechanism of these diverse allergic manifestations and the problem of resistance to liver extracts and to insulin

taking in consideration his own work and that of various American authors.

Therapy in case of allergic symptoms caused by the aforementioned drugs is described. In case of hepato-resistance, the author shows, by a personal example, that peroral treatment with extracts of stomach can be highly effective where injections of liver extract fail. On the other hand, an effective treatment of complete resistance to insulin is, up to the present time, completely unknown.

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From the State Serum Institute (Director: Jeppe Ørskov, M.D.) and the Blegdam Hospital (Chief: Professor H. C. A. Lassen, M.D.)

ON THE DEMONSTRATION OF COMPLEMENT-FIXING ANTIBODIES AGAINST SULPHONAMIDES AND SANOCRYSIN

EXPERIMENTAL AND CLINICAL INVESTIGATIONS

By

MALTHE JACOBSEN

(Copenhagen)

In the course of a broadly planned endeavour by various methods to demonstrate antibodies against low-molecular substances, especially sulphonamides, I have arrived at results with the complement-fixation reaction which I consider to be of interest.

That low-molecular substances after diazotisation and coupling to proteins may form antibodies directed against the coupling product, has been known since the fundamental experiments of Landsteiner and his co-workers. The demonstration of the antibodies is easy when the coupling product is employed as antigen (in anaphylaxis tests, by complement-fixation, or precipitation reaction); but it has not been possible by these methods to demonstrate antibodies when the pure low-molecular substance is used as antigen. Klopstock & Selter found that it was possible indirectly to show that the pure substances entered into a combination with the antibodies, for the reaction between the antibody and the coupling product could be inhibited if a solution of the pure substance were first added to the serum. This held good both of the precipitation test and complement-fixation. Although many attempts have been made, few workers have succeeded in demonstrating antibody in man or animals produced by action on the pure low-molecular substances (sulphonic acid compounds, atoxyl, salvarsan, sulphonamides etc.). Landsteiner & Jacobs report that in some few experiments on guinea-pigs immunized

by intracutaneous injections of arsphenamine they were able to produce an anaphylactic reaction by the intravenous re-injection of the same. Complement-fixing antibodies have never been demonstrated on experimental animals or on patients showing allergic reactions after treatment with sulphonamide. Only one work was able to report on complement-fixation with a low-molecular substance in serum from patients allergic to drugs, i.e. in some cases of salvarsan hypersensitivity, communicated by Ensbruner & Wendlberger. These authors were investigating some cases where the sensitivity could be transferred passively a.m. Prausnitz-Küstner, and some where this could not be done; they also examined some patients who had had slight, transitory erythema or severe dermatitis. It was found that when to the usual haemolytic system (sheep-blood corpuscles + amboceptor (inactivated serum from rabbits pre-treated with injections of guinea-pig kidney extract) + complement) they added together with the complement a certain concentration of salvarsan, haemolysis was inhibited: the result was an anticomplementary effect. The authors found that the strongest concentration of salvarsan which never had this effect was a dilution of 1 : 8,000 (exactly 1 : 8,192). The same effect was observed when salvarsan was added to the antigen in an ordinary Wassermann reaction with syphilitic or non-syphilitic patient serum: concentrations higher than 1 : 8,000 inhibited haemolysis, whereas lower concentrations had no influence on it. Then, if in the Wassermann test they used a saline solution of salvarsan instead of the usual antigen, they found, on testing with serum from patients with salvarsan dermatitis or other salvarsan reaction, that haemolysis was inhibited in much weaker concentrations of the salvarsan; the reaction could be titrated right down to a concentration of e.g. 1 : 1,048,576. They observed that this haemolysis-inhibiting effect ran parallel with the patient's clinical hypersensitivity. If the patient had a strong cutaneous reaction to salvarsan, the reaction was also strong; if the patient's hypersensitivity could be passively transferred a. m. Prausnitz-Küstner, the "complement-fixation" was strong too. If the patient's cutaneous reaction subsided, the "complement fixation" disappeared too. In the case of transitory ery-

thema, the reaction was transitory and weak, whereas it was more permanent and much stronger in the case of massive dermatitis. In order to show that the reaction was in fact due to hypersensitivity to drugs, the authors experimented with various control sera: from normal subjects, from syphilitics with positive or negative WR but with no sign of salvarsan hypersensitivity (including some who had never had salvarsan), from patients with fever (infectious diseases, antiluetic malaria treatment), and from patients with dermatitis not due to salvarsan or some other drug. They did not find the slightest inhibition of haemolysis with salvarsan 1 : 8,000. On the other hand it proved that the reaction was not specific, as it could be produced by many different "antigens", for example glucose, sodium arsenate and nickel sulphate, substances which were quite indifferent to the patient's hypersensitivity. Whenever there was a reaction with salvarsan, there was always a reaction with the other reagents. It was a feature common to the substances employed that every one, like salvarsan, down to a certain degree of dilution inhibited the ordinary haemolytic system, no matter whether human serum was added or not. The highest concentration that never influenced the haemolytic system differed for the various substances; for salvarsan, as already stated, it was 1 : 8,000; for solusalvarsan 1 : 160,000, solganal 1 : 16,000, myosalvarsan 1 : 500,000, and for nickel sulphate 1 : 16,000. Having regard to this reaction with many different substances the authors do not consider it proper to say that salvarsan behaves as a true antigen, but make use of the term "pseudoantigen". In their opinion, the explanation of the phenomenon they observed is that the "colloid-labile" serum from the hypersensitive patient has an effect on the substance which in advance, in a certain concentration, inhibits the complement in such a manner (or is so affected by the substance) that the complement-inhibiting effect becomes intensified many times over. Thus even if a specific reaction is out of the question, the authors attach importance to their discovery, it having been found, as already stated, that the reaction was to a high degree dependent on the other manifestations of hypersensitivity by the patient, and they employ the reaction, compared with the cutaneous reac-

tion and the outcome of the Prausnitz-Küstner reaction, as a criterion showing when it is proper to continue with salvarsan treatment.

This is the only work in which I have been able to find a report of positive findings with complement-fixation reactions in sera from individuals treated with the pure low-molecular substance and with the pure substance as antigen. In a number of works of a casuistic nature on hypersensitivity to sulphonamide there is a remark that attempts have been made to demonstrate the antibody, e.g. by complement-fixation, with no success.

OWN INVESTIGATIONS

METHOD

In my complement fixation experiments I employed the technique followed by the State Serum Institute for the gonococcus complement-fixation reaction and worked out by Martin Kristensen (1930). The entire test is carried out at pH 7.38. The haemolytic system consists of a 2.5 % suspension of sheep blood corpuscles to which is added an excess (three times the necessary quantity) of amboceptor (inactivated serum from rabbits immunized with injections of guinea-pig kidney emulsion). This mixture is called "sensitized blood". The serum to be tested is inactivated previously by standing for 30 minutes in a water bath at + 56°. The complement in the test is a fresh guinea-pig serum, its content of complement first being determined by a titration. (On this determination of the complement titre see Martin Kristensen's work). Here it will suffice to say that the titre must be found both for tests with antigen added and for tests without antigen, as owing to the presence of antigen it may often happen that complete haemolysis requires rather more complement than in a test without antigen. It is said that the antigen is self-inhibitory, i.e. that it possesses an anti-complementary property. For reliable results it is necessary to choose such a concentration of antigen that this self-inhibitory effect is the smallest possible or, preferably, is non-existent, that in other words practically the same amount of complement must be used in the tubes with antigen as in the control tubes without antigen. In the following results it is understood that the tests were so adjusted that this condition was satisfied. As some sera are self-inhibitory, i.e. without the presence of antigen they have an anti-complementary effect, every serum used in a test must be examined for that property. Therefore a control tube with serum + complement + sensitized blood is always included in the test for each serum, whereas the test proper consists of tubes

with serum + antigen + complement + sensitized blood. For the purpose of obtaining a quantitative expression of the content of antibody the method requires a titration of serum, a test being made of how far dilution can be carried and still give a reaction. If the serum is self-inhibitory the self-inhibition must also be titrated. On the subject of performing the titration and the reading of the reaction in degrees of haemolysis (haemolysis degree 10 = 100 % haemolysis, 6 = 60 % haemolysis, and so on), and on the conversion to degrees of strength, I would refer the reader to Martin Kristensen's work. Here I would merely say that the titration method requires that the quantity of serum in a tube is always equal to one third of the quantity in the foregoing tube. Furthermore, Martin Kristensen's conversion of the curve of the degree of haemolysis in the tube series is based upon the observation that the curve usually describes a course of a certain steepness, e.g. 2-8-10-10 or 0-4-9-10, but that sometimes there may be a more or less steep curve, for example 0-8-10-10 or 1-4-8-10. Reactions such as these cannot be converted to strength-degrees according to the usual system, but Martin Kristensen describes a method for the calculation of an approximately correct strength-degree, pointing out expressly that the method is arbitrarily chosen but that in practice it gives applicable results. Such an approximate expression for the degree of strength may be described as "read arbitrarily", and in the tabulation of my results I have marked it with an apostrophe before the figure. If the curve is still flatter, and especially if the same partial degree of haemolysis is observed in two successive tubes (e. g. 3-5-5-9 or the like), the reaction cannot even be "read arbitrarily" and must be described as unreadable. It will be seen later that in my experiments with sanocrysin I often found reactions of this kind.

When devising his system Martin Kristensen found that the same serum might show a certain variation in the degree of strength on different days (e.g. from 1 to 3 or from 10 to 12); he therefore introduced a system of control, by including every day a number of sera of known strength. When after the experiment he found the strength of these sera for this particular experiment, he calculated their deviation from the known and definitely established strength and inserted this plus or minus value as a correction on the strengths of the day's experimental sera. For example, correction of + 1 means that 1 must be added to all the values found, or in other words, that a serum giving a strength of 6 by direct reading actually has a strength of 77. In my experiments I was unable to obtain known, positive standard sera of this sort and therefore could not insert the correction.

The combination between antibody and antigen with the use of complement can proceed at various temperatures, and the intensity of the combination depends upon the temperature and time during which the process runs. For the gonoreaction at the State Serum Institute the fixation conditions are: 45 minutes at room temperature + 45 minutes in a water bath at 37°.

Martin Kristensen has shown that the fixation is more intense if it proceeds for about 20 hours at + 4°. While the reaction becomes stronger in this manner, the self-inhibition of the serum is also intensified. As I found very weak complement-fixations everywhere in my sera, I elected to allow fixation to proceed at + 4° throughout the night, i.e. for 18 or 20 hours. It was inconvenient that the self-inhibition simultaneously became more intense; because although self-inhibition can be judged to a certain extent by titrating it as already mentioned, it is by no means certain that, from a test in which perhaps a self-inhibition of strength 2 and a reaction of strength 4 have been found, the conclusion to be drawn is that the antibody reaction can be expressed direct by the difference between the two values. Nevertheless, even if in this example it cannot definitely be asserted that the reaction in serum is 2 or 4, it may be permissible to conclude that the test has shown that in serum there is an antibody, or at any rate a complement-inhibiting substance, which in effect resembles an antibody.

The circumstance that I gradually went over to "cold fixation" involved the drawback that I was unable to determine the complement titre before the test was set up, as of course the result of the titration could not be had until the day after. After a number of experiments, however, it turned out that the error in applying the complement titre determined by "warm tests" was practically insignificant, the two titres nearly always being identical. To make certain that the titre employed was correctly chosen I always accompanied the cold fixation with a complement titration, so that next day, together with the reading of the test, I could ascertain whether or not this had been the case. As I have said, in almost every instance there was agreement, and in my results I have rejected all the tests in which the titre employed differed to any marked degree from that found afterwards. In these tests I used a complement titre varying between 0.18 and 0.24, employing for the tubes a titre equal to or up to 0.02-0.04 higher than the titre used for the serum control. In order to control the mixture I included every day a "complement-antigen control". This consisted of three tubes containing 0.30-0.25 and 0.20 ml. complement-antigen-saline respectively, the volume in the last two tubes being made up to 0.30 ml. with saline; no serum was added, however. The tubes accompanied the main test with regard to binding and addition of blood. Then, if the quantity of complement used in the test was correct, these three tubes were found to have a haemolysis-degree of 10-9-7. If too much complement was used, the value might be 10-10-9 (or even 10-10-10), whereas conversely, in the case of too little complement there would be no total haemolysis in the first tube and correspondingly less in the other two. These controls (the titre determination and the said three tubes) showed that the antigen had no anti-complementary effect under the experimental conditions, nor on the whole any disturbing effect on the haemolysis.

ANTIGENS EMPLOYED

The following antigens were tested: *sulphanilamide* (p-aminobenzolsulphonamide), *sulphathiazol* (sulphanilamidothiazol), *alphasol* (α-(sulphanilylamidothiazol)-aethansolphonic acid sodium), *lucosil* (2(p-aminobenzolsulphonamido)methylthiodiazol), *sulphonamide-azo-protein* (with protein from horse or rabbit serum) (the latter preparations made up according to Landsteiner), and *sanocrysin* (nitrii auro thiosulphas).

When about to employ a new antigen in a complement-fixation test, two things must be established: 1) Has the substance in itself an ati-complementary effect? and 2) What concentration of the substance gives the best results?

Pure *sulphanilamide* is soluble in a water bath at 90-100° (also at pH 7.38) in a concentration of 5 ‰, but not in a much higher concentration. This solution is not self-inhibitory. 1 ‰ sulphanilamide gave practically the same results as 5 ‰, whereas weaker solutions gave no effect. I elected to use 5 ‰ as antigen in my experiments, although it is not impossible that the optimal concentration may have been somewhere between 5 and 1 ‰. Owing to the question of solubility I was precluded from examining whether stronger concentrations could be used.

With regard to *sulphathiazol,* what I have said about the self-inhibition and reactivity of sulphanilamide applies here too, solubility being ten times lower. Thus I found that both 0.5 ‰ and 0.1 ‰ reacted and at almost equal strength, whereas lower concentrations had no effect. In this case I chose 0.5 ‰ as the standard dose.

The possibility might be entertained that a still stronger solution of sulphonamide than the above saturated solutions of sulphanilamide and sulphathiazol would give still better results. With this in view I made some experiments with *alphasol.* This compound in solutions lower than 40 % slowly splits off sulphathiazol, which is precipitated, so that it was justifiable to assume that when added to the test tubes it would at a certain juncture give a supersaturated solution of sulphathiazol. It was also possible that alphasol in itself might act as an antigen, so

much the more as when immunizing animals against sulphathiazol I had employed injections of alphasol. However, the experiments showed that it was so highly anti-complementary in its effect as to be useless for the purpose.

With *lucosil* I found that a saturated solution of 0.5 ‰ was not anti-complementary; but as I was unable to find sera that would react positively to it (the sulphanilamide-positive rabbit sera referred to below were not tested against lucosil), I could not determine any optimal concentration. The more easily soluble lucosil-monoethanol was so markedly anti-complementary that it could not be used in the experiments.

Only few tests were made with *sulphonamide-azo-protein,* this being of little interest to the present investigations, the purpose of which was to find antibodies against the pure sulphonamides. Nevertheless I did test it and found that concentrations of 10 % and 1 % were totally anticomplementary, whereas 1 ‰ could be used. Experiments with 1 ‰ and 0.1 ‰ showed very little difference as to this as far as sulphanilamide-azo-protein was concerned, whereas sulphathiazol- and lucosil-azo-protein gave much stronger reactions with 0.1 ‰ than with 1 ‰.

As to *sanocrysin,* I found that concentrations of 3 ‰ and lower were useful. As will be shown later, sanocrysin as antigen presented special problems, as a result of which I was unable to determine any real optimal concentration.

While continuing to work with sulphanilamide and sulphathiazol I frequently encountered the peculiar phenomenon—also in experiments which, as far as the complement titre was concerned, were irreproachable and in which other sera showed quite normal behaviour—that a self-inhibitory reaction in the control tubes was abolished in the reaction series, in other words that in the control tubes there might be reactions such as 0-6-9-10 as compared with 3-8-9-10 in the reaction tubes, or 0-3-9-10 against 1-7-10-10. This is remarkable, because after the usual experience with complement-fixation tests one would expect to find the self-inhibition of serum undiminished in the reaction tubes. Thus, not only would it be wrong to say that there is a positive reaction with sulphanilamide, but in actual fact there

seem to be signs that sulphanilamide counteracts serum's own anti-complementary property (*anti-self-inhibitory? anti-complement fixing?*). For the purpose of examining this phenomenon more closely I made an experiment in which, to some of the State Serum Institute's routine gonococcus-complement-fixation tests, I added sulphanilamide or sulphathiazol in the usual concentration to the gonococcus antigen-complement solution. I then found exactly the same results as when using ordinary gonococcus-antigen without sulphonamide. Accordingly, these compounds have no antagonistic effect on complement-fixation between ordinary human gonococcus antibody and gonococcus antigen (12 sera of strengths 0-10 were tested), so that the observed effect cannot be anti-complement fixing in general, nor can it be an effect on haemolysis itself, but must rather be called anti-self-inhibitory, though this is not intended to suggest that the nature of the phenomenon has become much clearer.

SERA EMPLOYED

In my experiments I employed serum taken from patients by venous puncture with a dry needle, from rabbits by tapping an ear vein, and from guinea-pigs by heart puncture. After coagulation and retraction the coagulate was centrifuged off. Before use the serum was inactivated (i.e. freed of active complement) by standing it in a water bath for 30 minutes at 56°.

PROBLEMS OF SELF-INHIBITION

It is common knowledge that self-inhibition is sometimes found in normal sera, but that it rarely attains to any high degree. Some sera which, in the non-inactivated state, are self-inhibitory, are deprived of this property by inactivation, whereas conversely it may happen that non-self-inhibitory, active sera become self-inhibitory by inactivation. Serum from immunized rabbits is comparatively often self-inhibitory, but usually to a slight degree only. On these problems I would refer to Alice Reyn (1947),

who has discussed them at some length. When stored in the frozen state, non-inactivated serum may become self-inhibitory, in which case it usually will not lose that property on being inactivated.

In the course of my investigations of the behaviour of the complement fixation test during and after several series with rabbits (see Tables I and II) my attention was frequently caught by the curious circumstance that midway in a series of non-self-inhibitory sera from the same animal self-inhibition would suddenly appear in one or more sera. On going into the matter I found that the self-inhibitory sera were from the periods towards the close of a series (after 6-10 days' treatment) and the first 2-8 days after the end of the treatment. This phenomenon might be surmised to be due to antigen already being present in the sera. Because, if this is the case and simultaneously there is antibody, the possibility of an absorption of complement of course exists, in which case the observed self-inhibition is not genuine, for it is actually an expression of an antibody-antigen reaction. The fact that the explanation is not simply that the mere presence of sulphonamide in serum causes the reaction, is to be seen from the circumstance that it is not observed in sera from the first days, in which sulphonamide is presumably present, and also that, by the ordinary colorimetric examination, which covers concentra-

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ABBREVIATIONS USED IN TABLES

S — strength
Sc — strength in control tube
Sr — strength in reaction tube
sa — sulphanilamide
st — sulphathiazol
alph — alphasol
luc — lucosil
san — sanocrysin
sa-a-p(H) — sulphanilamide-azo-protein (prepared from horse serum)
'3 — a strength "read arbitrarily" as 3.
? — beside a haemolysis curve or a strength indicates an atypical lysis curve, presumably due to an unclean tube.
K.70 — Rabbit No. 70
M.70 — Guinea-pig No. 70
P.70 — Patient (experimental person) No. 70

TABLE

Table of complement-fixation results from K.70, K.72 and K.80

Treatment: May 5th-16th sulphanilamide 5 cg. × 2 daily or alphasol 21st-September 5th sulphanilamide 5 cg. × 2 daily or alphasol 2 ml. × 2 alphasol 2 ml. × 2 daily. November 29th-December 12th sulphanilamide

The date column shows the date of drawing the serum.

Treated Serum	Date	K. 70				K.	
		Control	sa 5 ‰	Sc	Sr	Control	sa 5 ‰
a	8/4	10	10-9-10	0	0	10	10-10-10
1/5 b	2/5	10	10-10	0	0	10	10-10-10
16/5 c	11/5	10	10	0	0	10	10-10-10
d	18/5	4	0-5-10	≥1	3½	0	0-0-2
25/5 e	23/5	10	0-1-10	0	'5	10	2-10-9
f	1/6	10	10-10	0	0	10	5-10
h	27/8	÷	÷			3-10	0-7-10-10
21/8 i	31/8	10-10	0-8-10-10	0	'2	6-10	0-5-10-10
5/9 j	4/9	10-10	10-10-10-10	0	0	3-10	0-1-9-10
k	6/9	9-10	0-8-10-10	0	'2	0-7	0-0-7-10
l	10/9	9-10	1-8-10-10	0	2	0.2	0-0-7-10
m	13/9	10-10	4-8-10-10	0	1	0-4	0-0-8-10
n	22/10	6-8	3-7-5-9	0	'2	8-9	3-9-7-10
31/10 o	6/11	0-0-7-10	0-0-6-10	5½	'6	0-0-8-10	0-3-9-10
p	7/11	8-6-9-10	1-7-9-10	0	'2½	9-9-10-10	9-9-8-10
q	8/11	6-6-9-10	4-8-9-10	0	'1	2-8-10-10	1-6-9-10
s	11/11	1-4-9-10	0-1-8-10	4	5½	1-8-10-10	1-8-10-10
t	12/11	5-9-10-10	1-5-10-10	½	3½	8-9-10-10	3-7-10-10
u	13/11	8-9-10-10	1-5-10-10	0	3½	8-10-10-10	2-8-10-10
17/11 v	14/11	1-7-10-10	3-4-9-10	2½	'3	3-9-10-10	1-8-9-10
x	15/11	÷	2-8-10-10		2	5-9-10-10	8-9-10-10
y	16/11	5-8-10-10	6-9-10-10	½	0	5-9-10-10	8-9-10-10
z	18/11	6-4-9-10	6-9-10-10	'0?	0	÷	÷
æ	19/11	1-2-6-10	1-5-8-10	'4½	'3½	4-6-10-10	5-8-10-10
ø	20/11	9-10-10-10	1-5-8-10	0	'3½	10-10-10-10	4-9-10-10
ba	21/11	8-10-10-10	2-7-10-10	0	2	8-10-10-10	8-10-10-10
bb	23/11	6-10-10-10	0-4-10-10	0	4	8-10-10-10	1-8-10-10
bc	28/11	6-10-10-10	2-8-10-10	0	2	10-10-10-10	9-10-10-10
29/11 bd	6/12	8-10-10-10	1-8-10-10	0	2½	1-8-10-10	2-7-10-10
12/12 be	11/12	2-6-7-10	2-6-9-10	'2	'2	8-9-10-10	5-8-10-10
bf	16/12	4-9-10-10	2-8-10-10	1	2	6-0-10-10	0-1-8-10
bg	18/12	2-7-10-10	2-7-10-10	2	2	1-0-8-10	0-1-8-10
bh	20/12	8-10-10-10	8-10-10-10	0	0	9-6-10-10	9-10-10-10
bi	27/12	7-9-10-10	7-9-10-10	0	0	7-9-10-10	7-9-10-10

I

treated with sulphanilamide, and K.95, treated with alphasol.
1 ml. × 2 daily. May 28th 15 cg. sulphanilamide or alphasol 5 ml. August daily. October 31st-November 14th sulphanilamide 5 cg. × 2 daily or 5 cg. × 2 daily or alphasol 2 ml. × 2 daily. All injections intraperitoneally. For the rest, see text.

72		K. 80				K. 95			
Sc	Sr	Control	sa 5 ‰	Sc	Sr	Control	st 0.5 ‰	Sc	Sr
0	0	10	10-10	0	0	10	10-10	0	0
0	0	10	10-10	0	0	10	10-10	0	0
0	0	10	10-10	0	0	10	10-10	0	0
≷3	8	8	0-3-9	0	4½	10	10-10-10	0	0
0	'2	10	5-10	0	½	10	10-10	0	0
0	½	10	9-10	0	0	10	10-10	0	0
1½	2½	9-10	5-8-9-10	0	'½	3-8	0-1-8-10	1½	5
0	3½	10-10	8-10-10-10	0	0	9-10	5 8-10-10	0	½
½	'5½	10-10	3-7-10-10	0	'11½	9-10	3-8-10-10	0	1½
'2½	'5½	10-10	8-10-10-10	0	0	9-10	2-7-10-10	0	2
≷5	'6	8-10	2-7-10-10	0	2	8-10	1-6-10-10	0	3
≷4	'5½	6-9	6-9-10-10	0	0	9-10	4-9-10-10	0	1
0	'11½	4-8	5-8-6-9	'1	'½	÷	÷		
'5½	4½	1-3-9-10	1-4-9-10	'4	'4	2-7-9-10	2-7-9-10	'2	'2
0	0	5-9-9-10	2-8-9-10	'½	2	4-8-10-10	2-9-10-10	'1	'2
2	3	1-8-10-10	4-8-10-10	2	'1	4-9-10-10	2-8-10-10	1	2
2½	2½	1-3-9-10	0-1-9-10	'4½	'5½	4-9-10-10	0-5-10-10	1	3½
0	'11½	0-1-8-10	1-3-8-10	5	'4	5-9-10-10	0-4-10-10	½	4
0	2	2-7-10-10	2-8-10-10	2	2	8-10-10-10	2-8-10-10	0	2
1½	2	3-8-10-10	4-9-10-10	1½	1	4-9-10-10	5-10-10-10	1	½
½	0	÷	10-10-10-10		0	÷	7-10-10-10		0
½	0	9-10-10-10	9-10-10-10	0	0	9-10-10-10	9-10-10-10	0	0
		2-6-10-10	7-10-10-10	'2	0	7-10-10-10	7-10-10-10	0	0
'1	'½	1-1-10-10	0-3-9-10	'4½	4½	7-10-10-10	6-9-10-10	0	0
0	1	9-10-10-10	8-10-10-10	0	0	9-10-10-10	5-9-10-10	0	½
0	0	8-7-10-10	6-10-10-10	'0	0	8-10-10-10	5-10-10-10	0	½
0	2	10-10-10-10	9-10-10-10	0	0	9-10-10-10	5-10-10-10	0	½
0	0	5-10-10-10	5-10-10-10	½	½	8-10-10-10	5-10-10-10	0	½
2	'2	3-9-10-10	1-8-10-10	1½	2	8-10-10-10	8-10-10-10	0	0
0	½	2-6-10-10	2-7-10-10	'2½	2½	8-10-10-10	8-10-10-10	0	0
?	'5	8-9-10-10	0-9-10-10	0	'2	9-10-10-10	7-10-10-10	0	0
'5?	5	0-4-10-10	0-5-10-10	4	3½	6-10-10-10	6-10 10-10	0	0
'0?	0	7-10-10-10	6-10-10-10	0	0	9-10-10-10	9-10-10-10	0	0
0	0	3-9-10-10	4-9-10-10	1½	1	8-10-10-10	10-10-10-10	0	0

tions from about 20 mg% to about 1 mg%, or from 0.2 ‰ to 0.01 ‰ (a slightly modified form of the method suggested by Marshall, Emerson & Cutting and by Eldahl, Joensen & Vermehren), I have never succeeded in demonstrating free or combined sulphonamide in the said sera. It would indeed be remarkable if antigen and antibody were present together in a serum and yet fail to combine until complement was added; one might suppose that this union together with the absorption of complement would already take place in the organism, where complement is present in advance. Nevertheless, it is possible that by the inactivation process, whereby complement is of course destroyed, the coupling product is broken down again and can once more fix the complement. Perhaps the phenomenon is connected with the circumstance, as Leftwich assumes, that the antigen is present in a special state in individuals who have been given sulphonamide for at least five days. We can scarcely reject the theory that there may be a connection between the phenomenon and the circumstance demonstrated by Tate & Klorfajn, that in an unsensitized person sulphonamide is excreted uniformly, whereas in a sensitized person (patients with sulphonamide dermatitis) the excretion was irregular so that after the termination of the treatment it could be seen that the excretion extended over several days and that in fact there were pauses sometimes when no excretion could be found. Tate & Klorfajn considered their finding to be evidence that in the sensitized organism the sulphonamide is bound to antibodies in the tissues, from which it was liberated irregularly. Their observation, however, might perhaps mean that in the blood stream the sulphonamide is present in a particular state which makes excretion irregular, perhaps coupled in some special fashion (different from the simple adsorption normally taking place). Finally, the phenomenon may reflect a simple unspecific self-inhibition such as that observable in immunized rabbits and no doubt due to colloidal peculiarities in the globulins. Whatever the explanation may be, the self-inhibition referred to never occurs until after six to ten days' treatment and, in typical cases, lasts only two to eight days after treatment is ended. That it is fortuitous seems to be precluded by the fact that the phenomenon

TABLE II

Table of complement-fixation results from K.103 which was treated with sulphanilamide, 5 cg. × 2 daily from Aug. 26th to Sept. 7th, Oct. 29th to Nov. 11th and Nov. 25th to Dec. 10th. The date column shows the date of drawing the serum. For the rest, see text.

		K. 103			
Treated Serum	Date	Control	sa 5 ‰	Sc	Sr
a	20/8	9-10	9-10-10-10	0	0
26/8 – 7/9 b	30/8	8-10	9-10-10-10	0	0
26/8 – 7/9 c	5/9	8-10	8-10-10-10	0	0
d	9/9	9-10	9-10-10-10	0	0
e	12/9	9-10	9-10-10-10	0	0
f	14/9	9-10	9-10-10-10	0	0
g	22/10	4-8	2-7-6-9	'1	'2½?
29/10 h	2/11	4-6-10-10	3-5-8-10	'2	'2½
i	4/11	5-7-10-10	5-9-10-10	½	½
j	5/11	0-0-0-8	0-1-3-9	'8½	'7
k	6/11	0-2-7-10	0-1-7-10	5	5½
l	7/11	3-5-9-10	2-4-9-10	'2½	'3½
m	8/11	3-6-10-10	3-3-8-10	'2½	'3
11/11 o	11/11	3-5-9-10	0-1-8-10	'2½	5
p	12/11	3-4-9-10	1-1-8-10	'3	'5
q	13/11	4-7-10-10	2-5-10-10	'1	'3
r	14/11	5-8-10-10	4-7-9-10	½	'1
t	16/11	6-7-10-10	7-8-10-10	0	0
u	18/11	6-4-9-10	5-7-9-10	'2?	'½
v	19/11	3-5-8-10	6-8-10-10	'2½	0
y	25/11	3-8-10-10	2-7-10-10	1½	2
25/11 – 10/12 z	4/12	7-10-10-10	5-10-10-10	0	½
25/11 – 10/12 æ	9/12	7-10-10-10	6-10-10-10	0	0
ø	12/12	6-9-10-10	6-9-10-10	0	0

occurs with distinct regularity during and after several treatment series of four batches of rabbits, in which only two batches were together in time, the other two being treated months later, and that it is to be observed on days when the experiments as regards the quantity of complement were successful. It should be observed, however, that the find is not made on all the rabbits tested, and that it does not necessarily occur during and after each treatment

TABLE

Table of complement-fixation results from rabbits previously treated with serum), Nos. K.9035-9042 and K. 9044-9053. They were given 2 ml. sub- March 17th. The sections thickly outlined are the reactions with homologous

Serum	Control	Sc	Sa-a-p(H) 1 ‰	Sr	Sa-a-p(H) 0,1 ‰	Sr
K. 9035, d	10-10-10-10	0	0-0-3-10	7½	0-0-3-9	7½
K. 9036, d	5-10-10-10	½	0-0-1-8	8½	0-0-1-8	8½
K. 9037, d	10-10-10-10	0	0-1-8-10	5½	0-4-9-10	4
K. 9038, d	9-10-10-10	0	0-0-3-9	7½	0-0-3-9	7½
K. 9039, d	10-10-10-10	0	0-0-1-8	8½	0-0-4-10	7
K. 9041, d	10-10-10-10	0	0-0-2-8	8	1-3-10-10	4½
K. 9042, d	9-10-10-10	0	0-0-3-9	7½	0-2-10-10	5
K. 9044, d	3-9-10-10	1½	0 2-8-10	5	1-2-7-10	5
K. 9045, d	4-9-10-10	1	1-4-10-10	4	1-4-10-10	4
K. 9047, d	9-10-10-10	0	0-1-10-10	'5	0-1-9-10	'5
K. 9048, d	1-4-10-10	4	0-1-8-10	5½	0-1-5-10	6
K. 9049, d	10-10-10-10	0	1-3-10-10	4½	2-4-10-10	'3
K. 9053, d	1-4-10-10	4	0-0-5-10	6½	0-0-5-10	6½

series of the same animal (see Table I). In some cases it is seen that the degree of haemolysis in the reaction tubes is lower than in the self-inhibitory control tubes, perhaps expressing a real antibody reaction; in other cases, however, one sees the aforesaid phenomenon that the degree of haemolysis in the reaction tubes is greater as an expression of an anti-self-inhibitory effect in the sulphonamide. We may perhaps be justified in supposing that this self-inhibition is connected with the formation of an antibody, having regard to the fact that it occurs most frequently and most markedly in those animals in which in sera from other periods we observe apparently regular complement-fixation reactions.

When repeating a test on a serum which had once shown a positive reaction and then was not self-inhibitory, I sometimes found that it had become self-inhibitory in the meantime. This self-inhibition occurring during the storage of an inactivated serum apparently was due to the tubes in which it was kept, though clean in a general sense, having not been so clean as is

III

sulphanilamide-azo-protein (horse serum) and sulphathiazol-azo-protein (horse cutaneously 22 times in the period from Jan. 8th to March 8th. Serum drawn antigen. For the rest, see text.

St-a-p(H) 1 ‰	Sr	St-a-p(H) 0,1 ‰	Sr	Luc-a-p(H) 1 ‰	Sr	Luc-a-p(H) 0,1 ‰	Sr
4-9-10-10	1	2-6-10-10	3	3-9-10-10	1½	1-4-9-10	4
4-9-10-10	1	1-5-10-10	3½	9-10-10-10	0	0-1-9-10	'5
8-10-10-10	0	4-10-10-10	1	1-8-10-10	2½	4-9-10-10	1
6-10-10-10	0	3-7-10-10	'2	3-8-10-10	1½	2-7-10-10	2
10-10-10-10	0	8-9-10-10	0	2-8-10-10	2	1-8-10-10	2
10-10-10-10	0	8-10-10-10	0	9-10-10-10	0	4-10-10-10	1
9-10-10-10	0	8-10-10-10	0	5-10-10-10	½	5-10-10-10	½
3-9-10-10	1½	0-3-9-10	4½	2-8-10-10	2	0-0-8-10	'5½
2-6-10-10	2½	2-2-8-10	'4	2-8-10-10	2	1-5-10-10	3½
2-6-10-10	2½	2-6-10-10	'2½	0-6-10-10	3	0-1-6-10	6
1-4-10-10	4	0-1-6-10	6	0-2-10-10	'5	0-1-5-10	6½
3-9-10-10	1½	3-9-10-10	1½	1-8-10-10	2½	1-1-10-10	'5
0-4-10-10	4	0-0-4-9	7	0-3-10-10	4½	0-0-2-9	8

necessary for storing sera. The tubes came from the hospital laboratory, where cleansing was scarcely up to the very strict standard observed at the State Serum Institute. If there is merely the slightest trace of cleanser (acid or alkali) on the dried and sterilized tube, it will make a serum strongly self-inhibitory in a short space of time. Finds of this kind are not included in my material.

RESULTS

1) *Complement-fixation reaction after pre-treatment with pure sulphonamides.*

a) *Rabbits.*

The rabbits treated with pure sulphonamides may be divided into four groups according to the nature and dosage of the treatment.

The first group of 11 animals (K.70-K.80) were immunized with sulphanilamide injected intraperitoneally, in four series of

14 days each, 5 cg. twice daily. Three months elapsed between the first and second series, three weeks between the second and third, and two weeks between third and fourth. Between the first and second series (12 days after the first series) a single dose of 15 cg. was given. The reactions from the three rabbits of this group which gave the best results (K.70, K.72 and K. 80) are reproduced in Table I. It appears from the table that during and immediately after the treatment self-inhibition occurs, but that as a rule it is lower in degree than the simultaneous reaction in the reaction tubes. As a rule this self-inhibition disappears after cessation of the treatment, whereas the reaction persists longer, as is particularly clear in K. 70. For this rabbit we see typical reactions of strengths up to 4 in sera e, i, k, l, m, n, ø, ba, bb and partly bc. The same is observable for K. 72 and K. 80, but scarcely so pronounced. The significance of the self-inhibition is discussed above. For the other eight animals of this group, self-inhibition was observable as in the case of the three just mentioned, but it was less marked and none of the reactions could be called positive.

The second group of 11 rabbits (K.90-K.100) received alphasol intraperitoneally in series exactly the same as Group 1 and in doses of 2 ml. twice daily. K.95 gave the best results, which are shown in Table I. They are analogous to those in Group I, but only slightly pronounced. The only positive sera were h, j, k, l and m, whereas ø, ba, bb and bc showed such a faint reaction (strength ½) that it is scarcely possible to attach any importance to it. Nevertheless, the fact that the faint reaction occurs in the very same sera as the positive reactions in Group I is strong evidence that there is really a positive complement-fixation reaction and not an unspecific accident. In the third injection series there were some rather strong reactions, the significance of which was made somewhat problematic by the simultaneous self-inhibitions, though in some cases these were much weaker than the reactions. As regards self-inhibitory reactions the other animals in the group presented weaker but analogous effects, whereas no really positive reactions could be found in any of them.

The third group (K.101-K.110) was treated with sulphanil-

TABLE IV

Table of the results of complement fixation with sanocrysin as antigen against human serum. Only the results with 1 ‰ solution are shown, these being identical with those found with 2 ‰. Sera from P.669 to P.687 came from patients treated with sanocrysin, whereas the others are from persons who were not given the drug. Many of the haemolysis curves are so "flat" that the reactions actually must be described as unreadable. Therefore in these cases the "force-read" strength is fallacious. It appears from the table that there is no essential difference between patient sera and control sera as regards the flatness of the curves and the occurrence of "positive" reactions. For the rest, see text.

Serum	Control	San. 1 ‰	St.	Serum	Control	San. 1 ‰	St.
P. 669a	10	9-9-10-10	0	P. 683a	10	5-8-9-10	'1/2
— b	10	5-4-6-10	'2	— b	10	4-7-9-10	'1
P. 670a	10	3-7-10-10	'2	— c	10	4-9-10-10	'1
— b	10	4-8-10-10	'1	P. 684a	10	4-8-10-10	'1
— c	10	4-8-10-10	'1	P. 685a	10	3-6-10-10	'1 1/2
— d	10	7-8-10-10	'0	P. 686a	10	6-8-10-10	'0
— e	10	6-9-10-10	0	— b	10	5-9-10-10	'1/2
P. 671a	10	4-6-7-10	'1	P. 687a	10	3-4-10-10	'3
P. 672a	10	6-7-8-9	'0	P. 19a	10	6-6-8-9	'0
P. 673a	10	7-8-10-10	'0	P. 20a	10	3-5-6-9	'2 1/2
— b	10	8-9-10-10	'0	P. 21a	10	7-7-7-9	'0
— c	10	6-7-9-10	'0	P. 22a	10	6-7-8-9	'0
— d	10	6-7-9-10	'0	P. 23a	10	7-7-8-9	'0
— e	10	5-9-10-10	'1/2	P. 24a	10	8-9-9-9	'0
— f	10	5-7-9-10	'1/2	P. 25a	10	6-8-9-9	'0
— g	10	6-7-10-10	'0	P. 187a	10	7-10-10-10	0
P. 674a	10	5-6-9-10	'1/2	P. 261a	10	3-6-8-10	'1 1/2
— b	10	6-8-10-10	'0	— b	7	6-10-10-10	0
P. 675a	10	4-6-9-10	'1	P. 262a	10	10-10-10-10	0
— b	10	4-7-10-10	'1	P. 263a	10	10-10-10-10	0
— c	10	5-7-10-10	'1/2	P. 264a	10	10-10-10-10	0
P. 676a	10	5-7-10-10	'1/2	P. 265a	10	10-10-10-10	0
— b	10	5-7-9-10	'1/2	P. 266a	10	10-10-10-10	0
P. 677a	10	10-10-10-10	0	P. 551a	10	10-10-10-10	0
P. 678a	10	5-8-10-10	'1/2	P. 700a	10	9-10-10-10	0
P. 679a	10	10-10-10-10	0	— b	10	5-6-8-9	'1/2
— d	10	10-10-10-10	0	P. 701a	10	10-10-10-10	0
P. 680a	10	6-8-10-10	'0	P. 702a	10	5-9-10-10	'1/2
P. 681a	10	8-10-10-10	0				
P. 682a	10	7-9-10-10	'0				
— b	10	8-9-10-10	'0				

amide intraperitoneally in three series of 12, 14 and 14 days with intervals of 7 weeks and 2 weeks. Every day the animals were given 5 cg. twice. The readings of the animal with the strongest reaction (K.103) are shown in Table II. As will be seen there, the reactions were not actually positive, but, as in the other groups, self-inhibition occurred during and after the treatment. The other animals in the group gave similar results, but less marked than those of K.103.

The fourth group (K.113-K.122) was treated with sulphanilamide in four series, 5 cg. being injected twice intraperitoneally on each treatment day. The first series comprised five injection days five days' intervals. After a pause of ten days came six treatment days with intervals of five days. The third series was one of daily injections for fourteen days, with the first injection five days after the end of the second series. Finally, in the fourth series there were daily injections for fourteen days, commencing fourteen days after the completion of the third series. In all these animals there were weak self-inhibition reactions during the treatment, but only one animal (K.119) gave two sera which could be said to show a really positive complement-fixation reaction with sulphanilamide, viz. serum æ and serum ø, which came from the fourth and fifth days, respectively, after the completion of the third series. Both sera give negative control tests (9-10-10-10) (i.e. no self-inhibition), whereas the reactions gave 0-8-10-10 with strength '2 and 1-4-10-10 with strength '3½.

b) *Guinea-pigs.*

Two groups treated with pure sulphonamide were tested for complement-fixation.

Group 1 (M.9002-M.9021) were immunized with sulphanilamide 1.25 cg. twice daily for fourteen days. A week later a subcutaneous depot of sulphanilamide in 2 % agar was made. Twelve days afterwards the animals were injected four times with 5 cg. sulphanilamide intraperitoneally at three days' intervals, and then 5 cg. daily for twelve days. Serum was taken before treatment began, midway in the first series, five days after the first

series, midway in the last series, and finally, a week after the last injection.

Group 2 (M.9022-M.9031) received alphasol intraperitoneally in doses of 0.5 and 1 ml. in exactly the same series as Group 1, and serum was taken on the same days during and after the treatment.

All sera showed negative reactions to sulphanilamide or sulphathiazol, and no self-inhibition was found in any of them.

c) *Patients.*

A total of 39 patients were tested, all of whom had manifested "allergic" complications during or after sulphonamide treatment, viz. 3 treated with sulphanilamide, 25 with sulphathiazol and 11 with lucosil. The sera dated from the period two to five days after the disappearance of the complications (exanthema or drug fever). In no case there was the slightest complement-fixation with the sulphonamide. Nor was self-inhibition found in any serum.

2) *Complement-fixation reaction after pre-treatment with sulphonamide-azo-proteins.*

a) *Rabbits.*

Two rabbits (K.9032 and K.9033) were treated with 5 ml. sulphanilamide-azo-protein (horse serum) (sa-a-p(H)) or sulphathiazol-azo-protein (horse serum) (st-a-p(H)), three injections of 5 ml. at intervals of three days. Serum taken six days after the final injection gave no complement-fixation with sulphanilamide or sulphathiazol.

Ten rabbits (K.9034-K.9043) were treated with sa-a-p(H), twelve injections of 2 ml. subcutaneously at three days' intervals and, after seventeen days' pause, the same dose daily for ten days.

Ten other rabbits (K.9044-K.9053) were treated in exactly the same manner with st-a-p(H). In both groups serum was taken nine days after the first series and again six and nine days after the last. All these sera were tested against sulphanilamide

and sulphathiazol. One or two weak self-inhibition reactions were found, but otherwise no positive reactions.

The last serum from these rabbits (serum d) was tested with sulphonamide-azo-protein(H) as the antigen in complement-fixation experiments. The results are reproduced in Table III. In the first place, it is remarkable that several sera from Group 2 (animals immunized with st-a-p(H)) were self-inhibitory in contrast to sera from Group 1. Next, it is observed that (as already stated), 1 ‰ of sa-a-p(H) give complement-fixation of practically the same strength, whereas 0.1 ‰ is better than 1 ‰ of st-a-p(H) and luc-a-p(H). Finally, we see that both groups of animals give the strongest reaction with the homologous antigen (the self-inhibition in Group 2 being duly considered), though luc-a-p(H) has an almost stronger effect on Group 2 than st-a-p(H). Accordingly, there was a certain degree of specificity. The experiment also shows that the reaction is not directed against the isolated protein fraction in the complex but against the whole complex, because in the former case an equally strong reaction would have been expected with all the antigens in all the tests, whereas actually there were wholly negative tests in the first group with st-a-p(H) and luc-a-p(H).

b) *Guinea-pigs.*

Two groups of guinea-pigs were tested.

Group 1 (M.9054-M.9063) were injected with sa-a-p(H), 0.5 ml. subcutaneously eleven times at intervals of three days.

Group 2 (M.9064-M.9073) were treated in the same manner with st-a-p(H).

Serum was taken nine days after the last injection and tested against sulphanilamide and sulphathiazol. In no case there was complement-fixation or self-inhibition.

3) *Complement-fixation reaction after pre-treatment with sanocrysin.*

a) *Rabbits.*

Tests were made with two groups of rabbits treated with sano-

crysin: one already infected with tuberculosis, one with healthy control animals.

Group I (K.9117-K.9126) were infected with an intravenous injection of 10 cg. Calmette vaccine. Nine weeks later they were given an injection of 3 cg. sanocrysin intravenously, and thereafter every fourth day four more injections each of 4.5 cg. Three of the animals died before the sanocrysin injections began; none of the remainder died in the course of the treatment.

Group 2 (K. 9310-K.9317) were injected—without previous infection with tuberculosis—with sanocrysin in exactly the same doses as Group 1. One of the animals died the day after the fifth injection.

Blood was drawn from all the animals of Group 1 before the Calmette injection. All surviving animals of both groups were bled just before the first injection of sanocrysin, and all survivors were bled again a week after the final sanocrysin injection.

In no serum there was the slightest complement-fixation with sanocrysin, or any self-inhibition reaction.

b) *Patients.*

A total of 39 sera were tested from 19 different sanocrysin-treated arthritis patients (P.669-P.687). Here the curious phenomenon was observed that the haemolysis curve in many cases was remarkably flat, values such as 3-5-8-10, 6-7-9-10 etc. being frequent. However, about half of the results were negative, with values of 10-10-10-10 or 8-9-10-10 or the like. There was no great difference whether 2 ‰ or 1 ‰ sanocrysin was used, whereas 0.1 ‰ always gave negative results. It was mentioned above that 2 ‰ and 1 ‰ show no antigen-self-inhibition, and this was verified by the present experiments, in that the complement-antigen controls were always in order as they ought to be. From what was said of the control tests with sanocrysin on serum from non-sanocrysin treated individuals and from the discussion of these findings, I should scarcely credit the results with any value as an expression of a regular antigen-antibody reaction, even if the "positive" tests were in decided majority in

the patient group compared with the control group (see Table IV).

4) *Control experiments: complement-fixation tests against sera from man and animals not previously treated with the antigen.*

a) *Sulphonamides.*

With pure sulphonamides I have tested 111 different sera from rabbits taken before treatment, 50 sera from untreated guinea-pigs and 9 sera from patients not previously treated with sulphonamides. Not the slightest reaction was ever observed.

b) *Sulphonamide-azo-protein.*

With sulphonamide-azo-protein I tested 5 sera from patients not previously treated with sulphonamide (or of course with sulphonamide-azo-protein). No reaction was observed in any of them. I did not test rabbit or guinea-pig serum with this antigen.

c) *Sanocrysin.*

With this drug I tested 72 sera from various rabbits without previous sanocrysin treatment. They all showed negative results.

I also tested 20 sera from untreated people. Here I found results strikingly like those obtained from testing sanocrysin-treated patients: there were many wholly negative, and many others showing a flat lysis curve such as 3-5-6-10, 5-6-7-9, 7-8-9-10 and similar; and in particular there were some which were "positive" in the same degree as sera from the patient material (see Table IV).

DISCUSSION OF RESULTS

As regards the method itself in the complement-fixation test, my results make it evident that in principle it is applicable, for it is possible to find such concentrations of the antigens that they do not inhibit the complement. In the course of the investigation it was found that the reactions observed were so weak that it was

advisable to allow fixation to proceed in the cold for about 20 hours, because this intensified the reactions. It was a drawback that simultaneously the self-inhibitory reactions were also intensified. The problems relating to self-inhibition have been discussed in the foregoing. It turned out that self-inhibition could occur while sera were in storage, and certain circumstances indicated that this was due to the use of tubes from which every trace of cleanser had not been removed. Again, I observed a self-inhibition in sera from rabbits which were under treatment or had just been treated. This phenomenon was so general throughout entire series treated and tested at different times that it cannot have been accidental. The problems connected with this particular matter have also been discussed above, and it will be seen that I have been unable to discover a satisfactory explanation of them.

Mention has been made of the fact that rather often the lysis curves did not coincide quite with those described by Martin Kristensen as typical, the result being that I had to "read arbitrarily" the strengths in these instances. This was particularly the case when sanocrysin was used as antigen, a matter which I shall examine more closely below.

On going through the results it is quickly seen that interest must be focussed on the rabbit material, i.e. the four groups K.70-80, K.90-100. K.101-110 and K.113-122. As was stated above and as appears from Tables I and II, there are five animals (K.70, K.72, K.80, K.95 and K.119) for which there is "something", and the question now is whether this "something" is to be taken for a genuine complement-fixation, whether there is an antigen-antibody reaction. In the first place there are quite a number of reactions in which there is no simultaneous self-inhibition, reactions which at first glance must seem to be "good enough". The feature common to them all is that they occur with a certain regularity from one rabbit to another in proportion to the treatment, occurring in the first ten to fourteen days after the treatment is completed. This fact alone must argue strongly that they are of importance. The reactions in every case are only weak and display a certain variation from one serum to another in the same animal. But, when for instance in K.70 sera ø, ba, bb

and bc we find a number of reactions of '3½, 2, 4 and 2, it should be remembered that I had no standard sera for stabilizing the experimental conditions. I have said that Martin Kristensen found that the same serum is capable of varying as much as two degrees of strength from one day to another, an expression of fluctuating, uncontrollable factors in the experiment, a circumstance which he mastered by including known sera every day, and adjusting the results accordingly. My findings lie within Martin Kristensen's variation, so that the fluctuation from one serum to another is no argument against the reaction being a genuine complement fixation. If we compare with the corresponding sera from K.72 we shall find strengths of 1, 0, 2 and 0. If anything it must be said that this justifies our confidence in the reaction, for here the variation agrees very closely with that of K.70 and thus must presumably be a fluctuation due to the aforesaid experimental variations. Looking at the self-inhibition reactions during the course of the treatment, we again find a marked regularity in their occurrence, a regularity which seems to preclude any question of fortuity. The problem has already been discussed. Naturally, we cannot attach decisive importance to a reaction in a serum that is self-inhibitory to almost the same degree; but there is one thing that suggests an expression of an antibody reaction: the aforesaid regularity in the time of its occurrence.

A comparison of the four groups one with the other shows that most positive reactions appear in Group I, whereas the others contain very few. The groups not being very large numerically, this preponderance in Group I may mean that there happened to be more good antibody formers in this than in the others, and we can scarcely draw conclusions as to whether sulphanilamide is a better antigen than sulphathiazol (alphasol), or whether one method of immunization is better than another. But in any case, it appears from the material that it requires intense treatment to produce a reaction. All in all it may be concluded that after the intense treatment of rabbits with pure sulphonamides I am able in some few instances to demonstrate the presence of a complement-fixing antibody in the blood stream.

Guinea-pigs treated in a similar manner with pure sulphonamides gave no reaction whatever.

Patients treated with sulphonamides and during treatment manifesting hypersensitivity to the drug gave no complement-fixation reaction with sulphonamide. The cause may perhaps have been that owing to the slow solubility of the antigens the antigen concentration was too low.

Rabbits and guinea-pigs treated with sulphonamide-azo-proteins gave no complement-fixation with pure sulphonamides; but in the sera tested from rabbits there was always a strong complement-fixation when sulphonamide-azo-protein was employed as antigen. This is not surprising, as we know from many previous works that these drugs are excellent antigens, and in fact my experiment was made more or less "as a matter of form". Mention has been made of the fact that the experiment showed that sera from the animals treated with the sulphanilamide preparation revealed a certain specificity, in contrast to those treated with sulphathiazol preparations.

As regards sanocrysin, I found that this drug as antigen in complement-fixation experiments with human serum behaved differently to the sulphonamides: the haemolysis curve was very flat and most of the reactions had to be read arbitrarily; indeed, many were unreadable. And whereas negative results were always obtained from the control tests on sera from untreated animals and people with sulphonamide, and on both treated and untreated rabbits with sanocrysin, I found some positive reactions on testing normal human serum against sanocrysin. Thus I am unable to say that the reactions I have found in patient sera after treatment with sanocrysin are an expression of the presence of an antibody, but must rest content to draw the conclusion that as antigen in complement-fixation experiments sanocrysin behaves in a particular manner which makes reading difficult and the definite demonstration of an antibody impossible.

SUMMARY

The object of the present work was to ascertain whether it was possible to demonstrate circulatory, complement-fixing antibody against pure sulphonamide in man and animals after pre-treatment with pure, uncoupled sulphonamide.

The author explains the method followed in the complement-fixation experiments and the problems arising out of the use of sulphonamide solution as antigen, especially certain problems of self-inhibition.

Of 42 rabbits which in four different series were given an intense preliminary treatment with pure sulphonamide (sulphanilamide or sulphathiazol), five showed distinct complement-fixation reactions in several different sera when the homologous sulphonamide was employed as antigen. Of much greater frequency than the typical complement-fixation reactions was self-inhibition during and after the treatment of the animals. The problems concerned with this phenomenon are discussed, though no definite explanation is arrived at.

For rabbits pre-treated with sulphonamide-azo-protein there was no complement-fixation with pure sulphonamide as antigen, whereas there was fixation (as expected) with the coupling product.

No complement-fixation was found for guinea-pigs pre-treated with pure sulphonamide or for patients who during a sulphonamide treatment had manifested "allergic" symptoms.

For rabbits, some tuberculous, others healthy, treated with sanocrysin, the author was unable to demonstrate complement-fixation with sanocrysin. When sanocrysin was employed as antigen the author observed results from sanocrysin-treated arthroitis patients and in serum from normal persons which are scarcely to be interpreted as an antibody-antigen reaction.

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ACTION DE L'HISTAMINE ET DES ANTI-HISTAMINIQUES DE SYNTHESE SUR LA PERMEABILITE CAPILLAIRE DE LA BARRIERE HEMATO-OCULAIRE

Par

B. N. HALPERN, L. GUILLAUMAT et S. CRUCHAUD

L'observation clinique (1) a montré que les antihistaminiques de synthèse sont doués d'un effet thérapeutique particulièrement efficace dans les formes d'allergie où les phénomènes d'altération de la perméabilité vasculaire prédominent. Il en est ainsi de l'urticaire, de l'œdème de Quincke, du rhume des foins, et de certaines migraines, affections qui relèvent toute d'une augmentation de la perméabilité capillaire. Halpern a eu l'occasion de démontrer en 1942, avec Arloing, Morel et Josserand (2) que l'Antergan est quelquefois doué d'une action considérable sur certains œdèmes peri-néoplasiques à allure fluxionnaire. On sait également que les antihistaminiques empêchent l'apparition de la papule de Lewis survenant à la suite de l'injection dermique d'histamine et qui est dûe à un accroissement de la perméabilité capillaire. Au cours du choc anaphylactique peptonique ou histaminique, l'augmentation la perméabilité capillaire se traduit par une hémoconcentration consécutive à une fuite du plasma à travers les capillaires devenus trop perméables. Or, l'action des antihistaminiques de synthèse sur ces syndromes est bien connue. D'autre part, l'action protectrice du Phenergan (3277 RP) sur l'œdème pulmonaire aigu expérimental du lapin, et sur l'albuminurie orthostatique que nous avons étudiés recemment, s'explique également par l'action de ce médicament sur la perméabilité capillaire. (3, 4, 5).

Tous ces faits nous ont incités à rechercher un test permettant

de mettre en évidence de façon quantitative l'action des antihistaminiques de synthèse sur la perméabilité capillaire.

Il nous a semblé que la mesure de la perméabilité de la barrière hémato-oculaire à la fluorescéine pourrait être utilisée dans ce but.

C'est en 1881 qu'*Ehrlich*, injectant de la fluorescéine dans les veines de lapin, constate son apparition dans l'humeur aqueuse (6). Elle franchit la barrière hémato-oculaire au niveau des capillaires de l'épithelium du corps ciliaire, en arrière de l'iris, puisqu'elle apparait derrière le bord pupillaire avant d'envahir l'humeur aqueuse qui remplit la chambre antérieure entre la cornée et le cristallin.

Pour observer cette fluorescence de l'humeur aqueuse, nous sommes, grâce à la lampe à fente, ou biomicroscope, mieux armés qu'*Ehrlich* ; un éclairage focalisé, plus ou moins intense, condensé en une fente lumineuse plus ou moins étroite, permet une véritable « coupe optique » des tissus transparents du pôle antérieur de l'œil que l'on examine avec un microscope binoculaire à prisme redresseur.

I y a même moyen d'apprécier en quelque sorte l'intensité de la fluorescence de l'humeur aqueuse en faisant varier l'intensité lumineuse de la lampe qui sert à la dévoiler. La proportion de fluorescéine est-elle faible, un ampérage assez fort sera nécessaire pour que l'observateur apprécie la nuance verte. L'humeur aqueuse est-elle au contraire, largement infiltrée par le colorant, il suffira d'une lumière moins vive ; ainsi, le seuil chromatique de la sensation de couleur verte déterminé en modifiant l'ampérage permet-il d'apprécier l'intensité de la fluorescénce cest à dire de la perméabilité de la barrière hémato-oculaire. C'est sur ces données théoriques qu'*Amsler* et *Huber* (7) ont mis au point en 1946 une technique simple que nous avons utilisé comme méthode de mesure dans nos expériences.

Recherches personnelles

I°) — TECHNIQUE

Le microscope cornéen employé est la lampe à fente de Haag et Streit (de Berne) dont le grossissement faible est de X9 environ. Entre la prise

de courant et la lampe sont intercalés un rhéostat et un ampéremètre gradué en dixièmes d'ampères, de 0 à 5 ampères. Contrairement à ce qui est nécessaire dans l'observation humaine, la lampe n'a pas besoin d'être survoltée pour donner un faisceau lumineux satisfaisant. Celui-ci est diaphragmé par une fente étroite et longue. L'angle d'incidence de la lumière est réglé à 25 °. L'observateur règle son microscope sur la zone d'humeur aqueuse intermédinaire à la cornée et à l'iris en avant du cristallin. C'est sur le fond noir de la pupille qu'apparaîtra, au bord du sphincteririen, la tache verte de la fluorescéine. Immédiatement après l'injection intra-veineuse, un chronographe est mis en marche et, de minute en minute, on observe attentivement l'humeur aqueuse et l'on note soigneusement le moment de l'apparition de la couleur verte. C'est alors qu'on diminue graduellement l'intensité du faisceau lumineux par l'intermédiaire du rhéostat jusqu'au seuil chromatique. L'ampéremètre indique alors un chiffre qui peut servir de mesure du degré de la fluorescence : la quantité de lumière est inversement proportionnelle à la quantité de fluorescéine contenue dans l'humeur aqueuse. La fluorescéine apparait toujours au niveau de la pupille, mais elle se mélange à l'humeur aqueuse de façon très variable, brassant tantôt les couches postérieures précristalliniennes, tantôt les couches antérieures retrocornéennes. Le premier point d'apparition une fois établi, il y a donc avantage à balayer la chambre antérieure d'un faisceau très allongé pour déterminer les autres points de la courbe. Celle-ci porte les temps en abcisses, et les dixièmes d'ampères en ordonnées.

II°) RESULTATS

Dans nos essais nous avons étudie :

1— la perméabilité de la barrière hémato-oculaire à la fluorescéine chez l'animal normal.

2— Les modifications de cette perméabilité sous l'influence de l'histamine.

3— L'influence des antihistaminiques de synthèse sur la perméabilité capillaire altérée par l'histamine.

Dans le cadre de chacun de ces groupes d'expériences nous avons étudiés deux données essentielles :

1— le temps d'apparition de la bande fluorescente,

2— l'intensité minimum d'éclairement nécessaire pour atteindre le seuil chromatique.

1) — *Etude de la perméabilité de la barrière hémato-oculaire chez l'animal normal.*

Nous avons utilisé pour ces essais des lapins de 2 kg 500. Les animaux convenablement fixés, reçurent par voie veineuse 2 cc de fluorescéinate de soude à 1 % en solution physiologique. On suivait ensuite à l'aide du chronomètre et de l'ampèremètre, minute après minute, les variations de la concentration de la fluorescéine dans l'humeur aqueuse pendant 20 minutes. Chaque

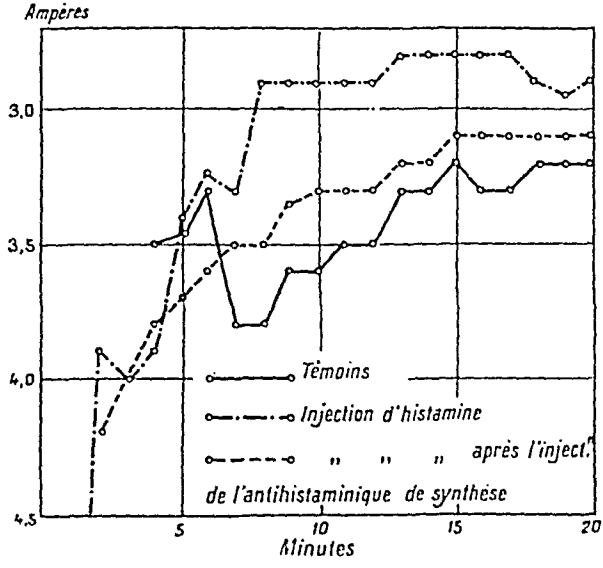

Fig. No. 1.

Etude de la perméabilité des capillaires du corps ciliaire chez le Lapin. Rythme de passage de la fluorescéine chez l'animal normal (témoins), chez l'animal ayant reçu 0 mgr 500 par kgr d'histamine et chez l'animal ayant reçu la même dose d'histamine mais qui a été préalablement protégé par une injection de phenergan (3277 RP). (Chaque courbe represente une moyenne de six experiences).

animal a été étalonné deux fois à 6 jours d'intervalle. Les chiffres obtenus sont relativement constants pour le même animal. (Fig. n° 1).

2) — *Effet de l'histamine sur la perméabilité de la barrière hémato-oculaire.*

L'action de l'histamine sur la perméabilité capillaire étant bien connue et démontrée nous avons eu recours à cette substance afin d'augmenter la perméabilité de la barrière hémato-oculaire à la fluorescéine.

Nous avons injecté 0,5 mgr/Kg de bichlorhydrate d'histamine diluée dans la même quantité de fluorescéine. La même manœuvre que précedemment a été appliquée. Sous l'effet de cette dose d'histamine, la concentration de la fluorescéine augmente nettement dans l'humeur aqueuse traduisant l'augmentation de la perméabilité capillaire.

Cette augmentation se manifeste le plus souvent vers la 8e minute et persiste jusqu'à la fin de l'expérience (*fig.* 1). Nous n'avons pas observé de variations notables du moment de l'apparition de la fluorescéine sous l'effet de l'histamine.

3) — *Action des anti-histaminiques de synthèse.*

Ces essais ayant démontré l'accroissement de la perméabilité de la barrière hémato-oculaire à la fluorescéine, sous l'effet de l'histamine, il nous a paru interessant d'étudier sur ce phénomène quantitativement mesurable l'influence des anti-histaminiques de synthèse. Nous avons utilié le N-dimethyl-amino-2-propyl-1-thiodiphenylamine, dont l'un de nous (Halpern) a démontré les propriétés anti-histaminiques. (8).

Le 3277 R.P. a été administré par voie hypo-dermique à 1 dose de 20 mgr/kg et trente minutes après, l'histamine et la fluorescéine ont été injecté dans la veine comme précedemment. Or, chez les animaux ainsi traités, le temps d'apparition de la fluorescéine ainsi que sa concentration dans l'humeur aqueuse, sont sensiblement égau aux chiffres trouvés dans les essais témoins (fig. 1).

Conclusion

Nous avons étudié la perméabilité des capillaires de la barrière hemato-oculaire à la fluorescéine à l'aide du biomicroscope oculaire. Après avoir établi les madalités de la pénétration de la fluorescéine dans l'humeur aqueuse de la chambre antérieure chez l'animal normal, nous avons pu constater que l'histamine accroît notablement la perméabilité du capillaires du corps ciliaire. Sous l'effet de l'injection préalable d'un anti-histaminique de synthèse, le N-dimethylamino-2-propyl-1-thiodiphenylamine

(Phenergan) l'action de l'histamine sur la perméabilité capillaire est annulée.

Clinique Médicale Propédeutique de l'Hôpital Broussais
(Professeur Pasteur Vallery-Radot)
et Hospice National des Quinze-Vingts. Paris.

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From the Out-Patient Department, The Hospital for Sick Children, Great Ormond Street, London.

ANTAGONIST DRUGS IN ASTHMA IN CHILDHOOD

By

A. P. NORMAN, M.B.E., M.R.C.P., D.C.H. and
F. WRIGLEY, M.R.C.S., L.R.C.P., D.I.H., M.P.S.

ATTENTION had already been directed to the possible use of antagonist drugs in the treatment of asthma in childhood by case reports of Dainow (1945) in which nicotinamide was suggested as an anti-histamine substance, and of Gate *et al.* (1942) who used synthetic anti-histamines before they were available in this country.

According to Dainow, cases of hay fever, urticaria and asthma were shown to have derived considerable relief from the administration of nicotinamide both parenterally and by mouth, and the possible use of a vitamin was thereby suggested. There was also the theory, based on comparison with animal experiments, that asthma might be due to the liberation of histamine, but the deduction that its cure might be possible by the use of drugs having a purely anti-histamine action, followed rather too easily. A search for antagonists having an action additional to an anti-histamine effect was further prompted by information from private workers that the more potent anti-histamine substances were not particularly effective against clinical asthma. A number of substances having antagonist actions was thus selected and a comparison of their effects on children attending the Out-Patient Department of the Hospital for Sick Children was made.

The children, whose ages ranged from 2 to 11 years, were usually seen at monthly intervals, and the number was limited in order that those chosen might be more carefully observed. Any child with a clinical diagnosis of asthma was included in the

series, but those in whom gross infection was present, and those whose mothers appeared unlikely to co-operate were excluded.

DIAGNOSIS

Diagnosis was based primarily on a history of typical symptoms in the child and secondarily on the clinical findings. A history of infantile eczema, the actual presence of Besnier's prurigo, a history of hay fever, nettle rash or persistent rhinitis, was considered to be confirmatory evidence that the child was suffering from asthma. A family history of asthma, eczema or hay fever, was not commonly found, a fact which contrasts with the conclusions of most authorities (Bray, 1937).

Intradermal tests for mixture of dusts and pollens were the only skin tests routinely performed, Bencard's Solutions A_1, A_2, B_4 and Control being used. Although Unger (1945) advised a full series of scratch tests in every case, it was not considered advisable to do this generally because of the dislike and fear produced in children by pricks and scratches.

A white cell count, total and differential, was routine at the commencement of treatment, and repeated if thought necessary. Whilst the presence of an eosinophilia was of interest as a finding frequently present in asthma, its absence was of no significance.

Radiographs of the chest and of the maxillary antra were taken of the majority of the children and where indicated antral washouts were carried out to exclude the presence of pus. In the long-standing cases the chest X-rays showed very frequently the appearance given radiologically the name of emphysema.

Selection of Antagonist Drugs.

The following substances were selected for use in the trial:—

(1) Nicotinamide (pyridine β-carboxylic acid amide).
(2) Allyl pethidine (allyl 1-methyl-4-phenylpiperidine-4-carboxylate hydrochloride).
(3) Benadryl (β-dimethylaminoethyl benzhydryl ether hydrochloride).
(4) S. 131 [β'-diethylaminoethyl, α, α-diphenyl-α(β''-dimethylaminoethoxy-acetate)].

Nicotinamide was selected because of Dainow's (1945) re-

ports. Allyl pethidine was considered because pethidine had been used in asthma, its spasmolytic activity being well known (Eisleb and Schaumann, 1939), and private reports stated that it had been found satisfactory. Pharmacological experiments revealed that the anti-histamine activity of allyl pethidine was greater than that of pethidine, and as its toxicity test revealed its MLD to be 155 mg./kg. subcutaneously in mice, it was decided to include it in our series. Benadryl was included because it was shown to have a fairly high histaminolytic activity, together with some spasmolytic action (this term is used in the sense of counteracting spasm produced experimentally by acetylcholine and barium chloride) and had been reported upon favourably (Logan, 1945). The MLD of this substance is 127 mg./kg. subcutaneously in mice (Gruhzit and Fisken, 1947). S. 131, an experimental compound, on which reports have not previously appeared, seemed pharmacologically to possess a fairly high spasmolytic activity, and also a histaminolytic activity somewhat less than that possessed by Benadryl. Toxicity tests have shown that the MLD is 150 mg./kg. subcutaneously in mice. (It is hoped that the full pharmacology of this substance and allyl pethidine will be published elsewhere). It was decided, with regard to these two latter substances, to determine whether clinical asthma responded better to a substance possessing a higher spasmolytic than histaminolytic activity, or vice versa.

Clinical Results with Nicotinamide and Allyl Pethidine.

Seven cases were treated with nicotinamide, 25 mg. being given three times a day. This substance had no effect. Allyl pethidine was given to 11 cases in a dosage of 2 mg. per lb. body weight per day in three divided doses. Eight of these cases were unrelieved, one appeared much improved and two slightly improved. Further trial was discontinued.

Report on Present Series.

In all, 43 cases were treated, and of these 17 were much improved during the whole of the period under observation. Some

cases only covered a short period, but are included because the improvement was very remarkable. One child whose attacks occurred on playing in new-mown fields was completely protected by taking one of the antagonist drugs described, but developed an attack on stopping the treatment.

No relief was obtained in 15 cases from Benadryl or S. 131. The remaining 11 seemed to be improved whilst receiving Benadryl or S. 131, but not markedly more than might be expected from routine palliative methods of treatment.

Five out of 7 cases treated with allyl pethidine without relief were much improved by Benadryl or S. 131, and 2 of the cases treated with nicotinamide without success responded to S. 131.

The dosage of Benadryl and S. 131 given was at first calculated at 2 mg. per lb. per day, but after the first few months of the trial this was increased to 3 mg. per lb. per day, generally in three doses, and never exceeding 50 mg. in a single dose. There were no ill effects, but two children seemed slightly drowsy after taking Benadryl, although not following S. 131.

Assessment of improvement was based on the mothers' and, where possible, the children's statements, that the attacks had ceased, the presence of not more than the slightest wheeziness, the ability to run about like other children and improvement in general health and appetite. An increase in weight was usually maintained and progressive. This was, in fact, observed in the majority of children who were much improved. The same constant gain in weight was shown by those few whose symptoms were due to naso-pharyngeal infection, and who responded to tonsillectomy and antral washouts, and who are not included in this group.

DISCUSSION

The results obtained show that asthmatic children are often relieved by the use of antagonists such as S. 131 and Benadryl. The clinical impression gained was that they were more benefited than would appear from the figures. There could be no doubt that this type of drug was effective, for contrasts between the

results obtained with S. 131 and Benadryl compared with those from allyl pethidine and nicotinamide were very striking.

Of those cases which failed to obtain any relief, some had an associated upper respiratory infection, and were later, to some extent, relieved by surgery and other methods. Several lived in such an unsatisfactory environment, either physically or psychologically, that failure to respond was hardly surprising, and others had failed to take the drug regularly. No case, which failed to respond to Benadryl, was relieved by S. 131.

Analysis of the series fails to reveal correlating factors in those cases responding to treatment, or in those failing to do so, and no differentiating factor between the two series could be found. The duration of the disease, the presence or absence of other allergic conditions and the clinical and laboratory findings were of no assistance, but the number radiologically classified as showing marked emphysema was very much higher in the group which failed to respond to therapy.

Although those cases which were improved by treatment might have reacted favourably to any form of therapy, it has been shown that many of them failed to respond at all to treatment with other drugs used. It can also be said that the large group which failed to respond, and also those which only showed slight improvement, are the groups which would fail to respond to any known treatment. This trial was begun not so much with the expectation of success, as with the object of evaluating some antagonist drugs in the treatment of asthma in childhood, and of recording for future guidance a comparison of results obtained with two drugs possessing different pharmacological properties.

CONCLUSIONS

The antagonist substances, Benadryl and S. 131, were shown to be of benefit in some cases of asthma, and of marked benefit in a few. The improvement in those children attending the clinic regularly was somewhat greater than is suggested by the records. Cases showing marked emphysema are likely to fall into the non-responsive group. The increase in the spasmolytic activity was not

shown to be of added value, though as said earlier, no side effects at all were reported with the substance having a greater spasmolytic activity. The histories of these cases again showed that tonsillectomy was of little value in asthma.

Further investigation is desirable on other antagonist substances, particularly if the action can be prolonged, but treatment should not be unduly continued in the absence of a positive result.

Acknowledgment.

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From the Neurological Department of the Frederiksberg Hospital, Copenhagen
(Chief Physician: T. Dalsgaard-Nielsen, M.D.)

ALLERGIC MENINGITIS AND CHRONIC ERYTHEMA MIGRANS AFZELII AFTER BITE BY IXODES REDUVIUS

By

T. DALSGAARD-NIELSEN and AAGE KIERKEGAARD

In a previous communication from this department a report was given of a case of Ixodes bite, presenting clinical features of polyradiculitis (4), which from the literature appears to be rather uncommon, as we have found it described hitherto only by Robert Bing (3). The patient recovered, but after a subsequent bite she had again symptoms of a lesion in the central nervous system—this time presenting the picture of fatal encephalitis with bulbar symptoms—which was taken to be of allergic character.

In the following an account will be given of a new case of a lesion in the central nervous system developing after the bite of an Ixodes reduvius.

Case Record.

The patient was a woman, 35 years old, with normal menstruation. Past history of the ordinary diseases of childhood, mild attack of diphtheria in youth, and puerperal fever at the age of 28. Otherwise she had always been well; in particular, she had not been liable to influenza-like diseases. The family history as well as her own history were negative as to allergic diseases.

On June 3, 1947, while visiting the island of Bornholm the patient was bitten by an Ixodes reduvius on the upper third of the anterior aspect of the right thigh. When she noticed the tick, she tore it off, but for some time after this the site of the bite

was swollen and red, infiltrated as a papule. After this, she was feeling well for a while, until 3 weeks after, when she noticed that a reddish, slightly elevated ring had developed round the site of the bite. This annular zone slowly spread centrifugally on the skin, with a smarting sensation, at the same time as the central area of the ring gradually bleached. The ring extended both distally on the extremity and up on the trunk, all the way up to the breast.

The first couple of weeks the body temperature was 38-39°.

After 2½ months the patient suddenly had pricking and cramp-like pains radiating from the gluteal region down in the entire right leg and, although in a lesser degree, in the left leg too. The pain in the left leg subsided within a couple of days, while it persisted on the right side and was complicated by lumbago-like pains. These pains now became permanent and, after a few days, the entire right lower extremity felt numb and tired.—There were no sphincter disturbances.

One week after the onset of the pains—*i.e.* 2¾ months after the bite—examination of the patient showed:

Mental habitus normal.

Appearance normal, with slight obesity.

Skull and cranial nerves: No abnormality.

Auscultation of the heart and lungs: Normal findings.

Abdomen normal, with normal reflexes.

Upper extremities, neurologically normal.

Lower extremities: In the lower part of Scarpa's triangle, on the right side, a large, glossy, scar (3 × 5 cm.) was seen. The surrounding skin looked normal, but at the level of the iliac crest above and at the transition between the middle and lower thirds of the leg a faintly red elevation of the skin was seen. Medially on the lower part of the leg there was a suggestion of hypesthesia. Otherwise normal neurological findings with moderately lively deep reflexes and absence of Lasègue's sign.

Romberg and gait normal.

Sedimentation rate: 21 mm./1 hour.

Total blood examination showed normal findings, with 2 % eosinophils. Wassermann reaction negative.

Spinal fluid: 41 leukocytes; 1-2 globulin, 13-14 albumin; normal pressure, with rise on Queckenstedt and abdominal pressure; negative Wassermann.

In the course of ten days, the pains in the leg subsided, but at the same time transitory radiating pains appeared along the right costal margin, and in the following month also transitory pains in the nape of the neck and left arm, besides along the left costal margin.

Even as late as 4½ months after the bite, the patient complained of paresthesias of the left arm and leg; and not until 5½ months after the bite was she again free from symptoms.

Epicrisis: The patient is a woman, aged 35, in whom the bite of a tick is followed after 3 weeks by the appearance of chronic erythema migrans. After 2¾ months a leukocytic meningitis is ascertained, taking a very benign course, with light radicular pains, in the beginning accompanied by slight fever (38-39°).

COMMENTS

While this combination of chronic erythema migrans Afzelii and meningitis following a bite by Ixodes reduvius has been described only in very few cases (Hellerström (9, 10) 1930, Gelbjerg Hansen (7, 8) 1945) the skin lesion alone has often been mentioned in the literature, first by Afzelius (1) (Sweden) in 1910 and by Lipschuetz (11, 12) (Vienna) in 1913.

The meningitis may be preponderantly lymphocytic—as in the cases reported by Hellerström and by Gelbjerg Hansen—or chiefly leukocytic as in our case. Fever (38-39°) appears to have been recorded only in the cases complicated by meningitis.

As to the *etiology*, Pawlowsky & Stein (14) have shown that extract of the salivary glands of Ixodes elicits a strong reaction on the skin of man, and that this reaction fails to appear if the extract is boiled; further the reaction is stronger than the one induced with extract from other parts of Ixodes.

Pathogenesis.—One might imagine this extract to contain an infectious agent—bacteria or virus—or an allergen that in certain

disposed subjects would elicit an allergic reaction from the skin and, less frequently, from the central nervous system too.

Looking into the infection theory, we find it conflicting with the following facts gathered from the literature and our case:

1. The disease develops in very few of the many persons bitten by Ixodes.
2. It has not been practicable to transmit the skin lesion to other subjects by means of extract from pieces of the skin attacked (Preininger (13)).
3. Cultures from these patients have yielded no particular bacterial growth (Hellerström (9, 10)).
4. Antibodies from several bacteria (B. abortus Bang, Gaertner, typhoid, paratyphoid A and B) have not been demonstrable in the patients, and the Wassermann test has turned out negative (Hellerström (9, 10)).
5. With a view to the conceivable possibility that here we might be dealing with a virus infection, we therefore had a sample of blood from our patient examined for the presence of antibodies for various viruses[1] (eastern and western equine encephalomyelitis, St. Louis encephalitis, choriomeningitis), and recently we received the report that the serum of our patient did not contain these substances.

While the infectious theory thus meets with considerable obstacles, there are some weighty arguments in favor of the allergic pathogenesis:

a. The disease may recur in the same patient after a new tick bite—as in the first-mentioned patient from this department. Analogously, Bode (4) has reported the cases of two sisters who both in three successive summers had an erythema following mosquito bites.

b. On microscopy of the skin infiltrations Gans (6) found numerous eosinophil cells, and Gelbjerg Hansen (7, 8) found eosinophilia in the blood.

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[1] This examination was obligingly performed by the National Institute of Health, Maryland, U.S.A.

c. On intradermal test with extract of Ixodes[2] our patient showed positive reaction of the normal skin, while 3 control subjects did not react. Nor was any positive reaction to the extract obtained in patients with meningitis of other etiology —kindly examined by Dr. Chr. Olsen, Chief Physician, Rønne Hospital, Bornholm.

d. While the reaction was distinctly positive on the normal skin of the patient, it was markedly weaker or even negative on those areas of the skin which had been attacked by the erythema; and this may readily be explained in keeping with the allergic theory as a phenomenon of desensitization.

In this connection it is to be mentioned that quite corresponding results with cutaneous tests were obtained by Hellerström (9, 10) (1933).

e. It is of decisive significance that in our case we were able to ascertain a positive Prausnitz-Küstner reaction, proving the presence of specific antibodies in blood of this patient.

So, as neither bacteria nor viruses—let alone their corresponding antibodies—have been demonstrated in this patient, while positive signs of allergy have been found histologically and by skin tests, with our present knowledge it will be justifiable to conclude that chronic erythema migrans Afzelii, with or without complicating meningitis, is an allergic reaction to the bite of Ixodes reduvius—presumably to a thermolabile substance from the salivary glands of the tick.

SUMMARY

In continuation of a previous report on a case of polyradiculitis after a bite by Ixodes reduvius an account is given of a case of leukocytic meningitis with radicular symptoms in connection with chronic erythema migrans Afzelii. Seroreactions for various virus diseases turned out negative. Skin tests with extract of Ixodes reduvius were positive on normal skin, negative on skin

[2] Prepared by Mr. P. C. T. Barfod, Ph.C., Frihavnsapoteket, Copenhagen.

areas which previously had been covered by the erythema. The Prausnitz-Küstner test turned out positive.

Like the erythema, the appearance of the meningitis is looked upon as an allergic phenomenon, taking a favorable course, as the patient was completely free from symptoms after 5½ months.

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